PREVALENCE OF CRYPTOSPORIDIUM SPECIES AND GIARDIA LAMBLIA INFECTION IN PATIENTS ATTENDING SIAYA DISTRICT HOSPITAL

KOSKEI KIPCHIRCHIR J, MBURU JANE, ODHIAMBO ROSE.

Department of Biological Sciences, Egerton University, P.O Box 536, Egerton, Kenya.
e-mail: jkkoskei61@gmail.com.

ABSTRACT

Introduction:

Objective: The study was conducted to assess the prevalence Cryptosporidium species and G. lamblia infections among patients attending Siaya district hospital.

Methods: A single stool specimen was collected from a total of 384 patients attending hospital laboratory with diarrhoea. The study was carried out over a period of two months covering January to March 2013. The specimens were processed for Cryptosporidium species and G. lamblia using ELISA (Giardia II/Cryptosporidium II) test and microscopy.

Results: Overall prevalence of G. lamblia was 20.3% while Cryptosporidium species was 7.6%. There were no significant difference observed in Cryptosporidium species and G. lamblia infection in relation to sex and age (p>0.05). Risk factors such as level of education, source of water and waste disposal were not significantly associated with Cryptosporidium species and G. lamblia infection (p>0.05). However, hand washing practice using soap was significant (p<0.05). Antigen detection by ELISA was more sensitive and showed high positivity in detecting cryptosporidium species (7.6 %) and G. lamblia (20.3%) infection compared to microscopy. There was significant difference between the two techniques (p<0.05). It was concluded that Cryptosporidium and G. lamblia are prevalent in patients attending Siaya district hospital with diarrhoea. There is need for public health education to be enhanced to discourage the adoption of risky practices. There is also a need for reliable diagnostic methods other than microscopy for Cryptosporidium species and G. lamblia stool tests in order to minimize the wide variation of the results in Siaya district and Kenya at large.

Conclusion: The study established Cryptosporidium species and G. lamblia infection was prevalent in Siaya district. There was also an association between hand-washing practice and infection. People who did not wash hands with soap before handling food had high prevalence of infection. In the present study ELISA technique had very good sensitivity and specificity hence should be used especially in patients presenting with diarrhea.

Key words: Giardia lamblia, microscopy, Cryptosporidium species, coproantigen, ELISA.

INTRODUCTION

Parasitic diseases are incriminated in causing more than 33% of global deaths of which intestinal parasitic infections are believed to take the majority share. Lack of safe drinking water and environmental sanitation contributes to more than 800 million cases of diarrhoeal diseases (Weitzel et al., 2006). Diarrhoea is a leading cause of morbidity and mortality among young children in low-income countries and kills an estimated 2.2 million people annually, 1.9 million being children (Gascon, 2000).

There are various aetiological agents of diarrhoea in endemic areas and include a wide variety of bacteria, viruses and parasites (WHO, 2009). Cryptosporidiosis and giardiasis are parasitic diseases that cause human diarrhoea and gastroenteritis worldwide and lead to significant morbidity and mortality, particularly in developing countries (WHO, 2005; Espelage, 2010). The causative organism of giardiasis is Giardia lamblia and several Cryptosporidium species for cryptosporidiosis. Of all the reported gastrointestinal protozoa parasites, Cryptosporidium species is the most morbid due to
its ability to cause severe diarrhoea in immunocompromised and even in immunocompetent individuals (Adamu, 2006). Generally cryptosporidiosis affects patients with several chronic health conditions that may have depressed their immunity. These include acquired immune deficiencies such as HIV/AIDS, diabetes and malnourished children (Awole, 2003; Adamu, 2006).

Giardiasis is the most common cause of parasitic gastro-intestinal disease and it is estimated that up to 200 million people are chronically infected with *G. lamblia* globally, and 500,000 new cases reported annually. The prevalence of the disease varies from 2% - 5% in developed to 20% - 30% in developing countries (Pereira, 2007). In sub-Saharan Africa the prevalence of diarrhoea caused by *G. lamblia* is between 2.6 - 4% while 5.7 - 8.4% is caused by *Cryptosporidium spp* (Hamer et al., 1998). In Kenya, mortality in children less than five years is due to diarrhoea in which water related diseases occupy a high proportion (Onyango and Ang’ienda, 2010). *Cryptosporidium* is the leading cause of enteric diseases especially in children in Kenya, accounting for 4% while *Giardia* accounts for 2% (Gatei et al., 2006).

**METHODOLOGY**

A single fresh stool sample was collected with labeled stool container from consulted subjects (n=384). The questionnaires were filled by the study participants and the stool sample was processed using methods stated below.

The Giardia II/Cryptosporidium II test is an enzyme immunoassay for the qualitative detection of *Giardia lamblia* cyst and *Cryptosporidium* oocyst antigen in human faecal specimen. It is used in faecal specimen from patients with diarrhoea to determine presence of *Giardia lamblia* and *Cryptosporidium* species in gastrointestinal infections. The technique was performed as recommended by Giardia II and Cryptosporidium II assay protocol. Optical density (OD) of test specimen and control (positive and negative) were read and recorded using 450nm filter. The cut off value was 0.150 (TECH LAB, 2006).

A direct wet mount method with normal saline (0.85% NaCL solution) was prepared and observed for the presence of motile trophozoite of *Giardia lamblia* under light microscope using 10X and 40X magnification. Lugol’s iodine staining was used to stain cysts. Using an applicator stick, about 1g of preserved stool sample was placed in clean 15ml conical centrifuge 7ml formalin. The sample dissolved and mixed thoroughly with applicator stick. The resulting suspension was filtered through a sieve (cotton gauze) into a beaker and the filtrate poured back into the same tube. The debris trapped on the sieve was discarded. After 3ml of diethyl ether was added to the mixture and hand shaken, the content was centrifuged at 2000 rpm for 3 minutes. The supernatant poured away and the tube replaced in its rack. Iodine stain preparation was made from the sediments. Finally the entire area under the cover slip was systematically examined using x10 and x40 objective lenses for cysts of *G. lamblia* (WHO, 1991).

For detection of *Cryptosporidium* oocyst, direct and concentration smears were prepared. Fresh faecal sample was collected from patients and thin smears prepared, air dried, fixed with methanol for 5 minutes and stained by Zeihl-Neelsen technique, and the same procedure was used for smears prepared after concentration. In this technique the slides were cleaned with Carbol Fuschin for 30 minutes and thereafter, they were washed with tap water. The slides were decolorized in acid alcohol for 1 minute and counter stained with methylene blue for 1 minute. Finally the stained smears were examined microscopically using x100 magnification for oocysts of *Cryptosporidium spp* (WHO, 1991; NHS, 2010).

**RESULTS**

Three hundred and eighty four (384) patients presenting with diarrhea were examined for *Cryptosporidium species* and *G. lamblia* infection in Siaya district hospital. Out of these number, protozoa etiologic agents of diarrhea was identified in 107 (27.9%). A total of 78 (20.3%) were positive for *G. lamblia* infection while 29 (7.6%) had *Cryptosporidium* species (Table 1).

| Table 1: Prevalence of *G. lamblia* and *Cryptosporidium species* infection. |
Out of the 384 study subjects, 181 were males and 203 were females. The prevalence of *G. lamblia* infection in female subjects was higher 46 (22.7%) than males 32 (17.7%). Male subjects showed higher prevalence in *Cryptosporidium species* infection 19 (10.5%) while females recorded 10 (4.9%). However, the sex of the subject did not show any statistical differences on the likelihood of *G. lamblia* and *Cryptosporidium* infection  \( p > 0.05 \).

The results of the study show that infection occurred in all the age groups. Among the 80 patients aged 0-9 years, 29.1% and 17.7% were positive for *G. lamblia* and *Cryptosporidium species* respectively. Among the 118 patients in the 10-19 year category, 21.9% were positive for *G. lamblia* and 7.6% for *Cryptosporidium species*. Patients aged 20-29 years had a prevalence of 17.3% and 5.3% for *G. lamblia* and *Cryptosporidium species* respectively. Among the 46 patients aged 30-39 years, 13.0% were found to be positive for *G. lamblia* and 4.4% for *Cryptosporidium species*. Those between 40 and 49 years old who numbered 35 had a prevalence of 20.0% for *G. lamblia* and 2.9% for *Cryptosporidium species*. Patients aged 50 years and above had a prevalence of 13.3% for *G. lamblia* and 6.7% for *Cryptosporidium species*. The difference in the prevalence of *G. lamblia* and *Cryptosporidium species* among different age groups was not statistically significant \( p > 0.05 \).
Majority of patients who tested positive for both Cryptosporidium species and G. lamblia who numbered 48 (12.5%) used well/boreholes water for drinking and cooking followed by those using rivers or streams 26 (6.8%), dams/ponds 16 (4.2%), and lake 15 (3.9%). The lowest prevalence 2 (0.5%) was observed in patients where tap water was used. There was no significant association between G. lamblia and Cryptosporidium species infection and source of water (p>0.05).

Figure 3: Association of Cryptosporidium species and G. lamblia infection and water source. (n=384)

Prevalence of G. lamblia and Cryptosporidium species was higher 81 (21.1%) in those who have primary education, followed by secondary 14(3.7%) and patients with no formal education 9 (2.3%). The least was those with post-secondary education 3(0.01%). However, there was no association between Cryptosporidium species and G. lamblia infection and level of education (p> 0.05).

Figure 4: Association of Cryptosporidium species and G. lamblia infection with level of education. (n=384)

The epidemiological data revealed that out of the 107 who were positive for the protozoa parasites, 91 (85.1%) did not wash their hands with soap before handling food while 16 (14.9%) did (Figure 8). There was strong association between hand washing practice and G. lamblia and Cryptosporidium species infection in this study p< 0.05.)
Figure 5: Association between hand washing practice before handling food Cryptosporidium species and G. lambia infection. (n=107)

A total of 384 patients requested to participate in this study responded to the item of method of disposing faeces. Out of the 107 respondents who were positive for the two parasites, majority, 56 (52.3%) used bush while 39 (36.5%) used pit latrines. Respondents who used flush toilets were the least 12 (11.2%). However, there was no association between mode of human disposal and G. lambia and Cryptosporidium species infection (p >0.05).

Figure 6: Mode of human waste disposal
(n=107)

Cryptosporidium species was detected in 29 (27.1%) patients using ELISA technique and 20 (18.7%) by microscopy (Table 3). Comparison between microscopy and antigen detection method revealed that 9 patients were positive by antigen detection technique and negative by microscopy. G. lambia was detected in 78 (72.9%) patients using ELISA method and 37 (34.6%) by microscopy. Comparison between microscopy and antigen detection technique also revealed that 41 (38.3%) were positive by antigen detection and negative by microscopy (Table 4). ELISA method had sensitivity of 100% and specificity of 100% in detecting the two parasites. The sensitivity of microscopy in comparison to ELISA was 65% and 47.4% in detecting Cryptosporidium species and Giardia lambia respectively (Table 5). The study revealed that there was a significant difference between the two techniques in detecting G. lambia and Cryptosporidium species (p< 0.05).

Table 3: Cryptosporidium species and G. Lamblia positivity by Elisa and microscopy methods (n= 107).

<table>
<thead>
<tr>
<th>Method</th>
<th>Positive sample</th>
<th>Prevalence (%)</th>
<th>Positive samples</th>
<th>Prevalence (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Microscopy</td>
<td>20</td>
<td>18.7</td>
<td>37</td>
<td>34.6</td>
</tr>
<tr>
<td>ELISA method</td>
<td>29</td>
<td>27.1</td>
<td>78</td>
<td>72.9</td>
</tr>
</tbody>
</table>
Table 4: Comparison of microscopy and ELISA methods for detection of Cryptosporidium species and G. Lamblia (n= 384).

<table>
<thead>
<tr>
<th>ELISA</th>
<th>Cryptosporidium species</th>
<th>G. lamblia</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. positive</td>
<td>20</td>
<td>37</td>
</tr>
<tr>
<td>No. negative</td>
<td>9</td>
<td>306</td>
</tr>
<tr>
<td>Total</td>
<td>29</td>
<td>347</td>
</tr>
</tbody>
</table>

Table 5: Sensitivity and specificity of microscopy in comparison to ELISA in the diagnosis of Cryptosporidium species and G. lamblia

<table>
<thead>
<tr>
<th>Parasite</th>
<th>Microscopy</th>
<th>ELISA</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Sensitivity (%)</td>
<td>Specificity (%)</td>
<td>Sensitivity (%)</td>
</tr>
<tr>
<td>Cryptosporidium species</td>
<td>69</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>G. lamblia</td>
<td>47</td>
<td>100</td>
<td>100</td>
</tr>
</tbody>
</table>

DISCUSSION

Infectious intestinal diseases are often self-limiting and in developing countries they are usually treated without proper diagnosis or information about etiology. However, information about pathogenic organism is very important for proper treatment, for monitoring trends as an early warning for identifying outbreaks and for introducing control measures and creating health policies.

The current study revealed that G. lamblia and Cryptosporidium species are prevalent among patients presenting with diarrhoea in Siaya district hospital. This finding was higher than those observed by Chunge et al., (1992), which was 2.7% for Cryptosporidium species and 3.8% for Giardia lamblia. The reason could be due to the fact that studies cited above used microscopy to demonstrate cysts of G. lamblia and oocyst of Cryptosporidium species in stool samples. The prevalence was also higher than what was reported by Gatei et al., (2006) which was 4% for Cryptosporidium species. However, it was lower than the study reported in Mbagathi hospital (Mbae et al, 2013). The prevalence recorded in this study was similar to those observed in Ethiopia by Tigabu et al., which was 8.1%.

All the age groups were infected with the protozoan as revealed in the study. The prevalence decreased with increase in age group but as patients advanced in age the prevalence also increased. Age specific prevalence of Cryptosporidium species and Giardia lamblia in the present study showed that those aged 0-9 years had higher prevalence than what was reported in Ethiopia by Tagabu et al., (2010) who reported a lower prevalence of 26.6% and Ayalew (2006) who observed a prevalence of 38%. The difference in infection prevalence of the two protozoa parasites between age groups in the present study is in agreement with what was observed in Uganda by Tumwine et al., (2003). This finding therefore suggests that Cryptosporidium species and
Giardia lamblia detection rate can vary depending on the age of the patient.

In the present study the prevalence of G. lamblia infection was higher in females than in males. This is in agreement with what was observed by Tagabu (2010) in Ethiopia. The possible explanation could be because of increased chance of exposure of females to contaminated water as they are engaged in fetching water for use at home as is the case in most family set up in Kenya. Cryptosporidium species infection was higher in males than females in the present study. The current study is in agreement with a study conducted in Ethiopia by Adamu (2006). This could be due to the exploratory nature of male children.

Access to clean water, good human waste disposal practice, high level of education, and good hand washing practice before meals are factors that could play a major role in control and prevention of giardiasis and cryptosporidiosis in Siaya district. The prevalence of these protozoa infection was high in those who used well/ boreholes than those who used water from other sources. Subjects who used bush as mode of human waste disposal was higher than those who used pit latrine, and flush toilets. Similarly, those who had primary level of education had higher prevalence than those who had no formal education, secondary and post-secondary. However, the present study showed no statistically significant association was found between the above mentioned risk factors and the acquisition of cryptosporidiosis and giardiasis. This study is in agreement with the report in India by Ajjampur et al (2007).

The prevalence was also higher among those who did not wash hands with soap before handling food than those who did. This study is in agreement with what was reported by Hoque et al (1995) and the study done in Malawi by Morse et al (2008). There was strong association between hand washing practice before handling food and the protozoa infection p< 0.05.

In the present study two different techniques used in the diagnosis of Cryptosporidium species and G. lamblia were evaluated. Comparative analysis of the two methods showed that both G. lamblia and Cryptosporidium species could be detected by microscopy and antigen detection technique. The antigen detection method (ELISA) gave the highest number of positive samples for Cryptosporidium species and G. lamblia. Microscopy on the other hand gave lower number of positive samples for both Cryptosporidium species and G. lamblia. The result obtained in this study was in agreement with the report by Haque (2007).

Comparison between microscopy and ELISA revealed that 8.3% of the patients were positive by antigen detection and negative by microscopy for Cryptosporidium species and 38.3% for G. lamblia. It was established that sensitivity of microscopy in this study was lower than ELISA technique. However, the results obtained did not agree with the observation by Youn et al (2009).

CONCLUSION

This study was performed to determine the prevalence of parasites causing diarrhoea in Siaya district hospital. It established that Cryptosporidium species was prevalent and presence of this parasite in patients with diarrhoea is an indication that cryptosporidiosis exists in this area. It was established that the prevalence of this parasitic infection was higher in males than in females. The study also established that patients aged 0-9 years had higher prevalence of Cryptosporidium species infection. Prevalence of G. lamblia was found to be high in this area and infection in females was higher than in males. However, the finding of this study was subject to one limitation; the data do not include immune status of patients.

The study revealed that there was strong association between hand washing practice and infection. There was a strong link between hand washing practice using soap and reduced G. lamblia and Cryptosporidium species infection. This shows that washing hands with soap can reduce Cryptosporidium species and G. lamblia infection. The use of soap facilitates detachment of pathogens from hand surface and therefore this practice should be promoted as one of the intervention method. Other risk factors such as level of education, source of drinking water and mode of human waste disposal had no association with cryptosporidium species and G. lamblia infection.

Microscopy has been the tool available for the detection of Cryptosporidium species and G. lamblia. However, proper diagnosis depends on qualified technicians and technologists. In developing countries, where resources are limited, this proves to
be difficult and misdiagnosis can significantly impact on patient care. In the present study ELISA technique had very good sensitivity and specificity while microscopy was very specific but less sensitive method for the laboratory detection of oocysts of Cryptosporidium species and G. lamblia cysts. This suggests that cases of cryptosporidiosis may be missed in patients who have diarrhoea if microscopy alone is employed.

ACKNOWLEDGEMENT
I would like to thank the management of KEMRI Kisumu for allowing me to use their facilities and Dr. Peter Odada Sumba for his invaluable advice during the entire period of my research study and Dr. Jane Mburu and Prof. Rose Odhiambo for their guidance. My sincere thanks go to KEMRI employees in microbiology department in particular David Omar Chemjor for help with laboratory reagents.

REFERENCES


