Coproantigen versus microscopic examination for diagnosis of Gardia Lamblia and Entameba Histolytica infection in children in Banha Teaching Hospital

By

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ABSTRACT

Background: *Giardia lamblia (G. lamblia) and Entamoeba histolytica (E. histolytica) are of the most common protozoan enteric pathogen in humans. Their laboratory diagnosis mainly consists of direct microscopic examination of stool specimen for trophozoites and/or cysts. However, due to intermittent fecal excretion of the parasite, the patient may be misdiagnosed and infecting others. Recently, immunological testing of stool (coproantigen) for E histolytica G lamblia has been reported as a more sensitive mean for their diagnosis.*

Aim of Work*: Evaluation of the efficacy of coproantigen detection by ELISA technique in comparison to direct microscopical examination for diagnosis of Entamoeba histolytica and/or Giardia lamblia.*

Patients and Methods: *One hundred ten children were included in this simple comparative study. They were divided into three groups:*

- Group (1) included 40 children with dysentery their stools were examined for E. histolytica,

- Group (2) included 40 children with abdominal complaints their stools were examined for G. lamblia,

- Group (3) included 30 normal children their stools were examined for detection of both E histolytica and G. lamblia

Results: *Group (1) by microscopic examination 35% were positive for trophozoites and/or cysts and 65% were negative while by Coproantigen test 47.5% were positive and 52.5%were negative[sensitivity 73%, specificity 100% and NPV 80.7%].*

Group (2) by microscopic examination 27.5%were positive for trophozoites and/or cysts and 72.5% were negative while by Coproantigen test 37.5% were positive and 62.5% were negative [sensitivity 73.3%, specificity 100%,PPV 100% and NPV 86.2%]

Group (3) For E. histolytica :by microscopic examination 30% were positive for trophozoites and/or cysts and 70% were negative while by Coproantigen test 23.3% were positive and 76.7% were negative[sensitivity 100%, specificity 91.6% ,PPV 77.7% and NPV 100%]

For G. lamblia: by microscopic examination 16.6% were positive for trophozoites and/or cysts and 83.4% were negative (while by Coproantigen test 26.6% were positive and 73.4% were negative [sensitivity 62.5%, specificity 100% , PPV 100% and NPV 89.2%].

***Conclusion:** Coproantigen ELISA technique is a rapid and effective method with high sensitivity and specificity for diagnosis of E. histolytica and G. lamblia*

INTRODUCTION

Intestinal parasitic infections (IPIs) have high prevalence around the world, especially in developing countries **(Safi et al., 2016)**. G. lamblia and E. histolytica are of the most common protozoan enteric pathogen in humans. G. lamblia has a prevalence ranging from 20% - 30% in developing countries and 2% - 5% in developed countries that affected about 200 million individuals throughout the world (Kurdova et al., 2007). In addition, E. histolytica, affects about 180 million individuals worldwide. It is highly endemic throughout poor communities in the tropics and subtropics **(Stauffer and Ravdin, 2003)**. The genus Entamoeba contains many species of which E. histolytica, E.dispar, E.coli. E.hartmanni and E. moshkovskii are found in the human intestinal tract. Cysts of E. histolytica, E. dispar, and E. moshkovskii are morphologically indistinguishable by microscopic examination **(Van Den Broucke et al., 2018)** but the species are biochemically and genetically different. The first is a potential pathogen, while the latter is a non-pathogenic **(Fotedar et al., 2010)**.

G. lamblia infection leads to malabsorption and diarrhea, but most often it occurs asymptomatic. Infections in children have a negative impact on growth and development **(Lane and Lloyd, 2002)**.

Detection of G. lamblia is traditionally performed by microscopic examination of stool specimens .The sensitivity of morphodiagnostic technique is approximately 46% on a single step due to the intermittent excretion of cysts and at least three faecal samples have to be obtained over a 3-5 day period to achieve 94 % accuracy, so it is time consuming, and requires high degree of client compliance **(Papini and Cardini 2006)**.

Examination of duodenal aspirate is invasive, costly and uncomfortable for the patient. Serum anti-Giardia antibody detection fails to distinguish current from past infection **(Noor et al., 2014)**.

In most cases E. histolytica infection is asymptomatic. Symptomatic cases may suffer from either intestinal or extra-intestinal infections, manifested as local tissue destruction by the ameba trophozoites **(Haque et al., 2002)**.

In most cases, the diagnosis of E. histolytica is based on microscopic detection of the parasite in both fresh stool samples and in culture at 37°C. However, due to morphological similarities between E. histolytica and the non-pathogenic

E. dispar these tests may be misleading **(Jackson, 1998)**.

Culture is more sensitive than microscopy and isoenzyme analysis of cultured amebae enables the differentiation of E. histolytica from E. dispar. However, amebic cultures and isoenzyme analysis require a week to complete and are negative in many microscopy-positive samples **(Haque et al., 1998)**.

Recently, immunological testing of stool for E histolytica or G lamblia coproantigen has been reported as more sensitive mean for their diagnosis **(Shahat et al., 2017)**.

AIM OF THE WORK

Our study aimed to compare the specific coproantigen level and microscopic examination as tools for diagnosis of G. lamblia and E. histolytica infection in children.

PATIENTS AND METHODS

This simple comparative study was performed on 110 children from Banha Teaching Hospital during the period from March to Sept 2017 .Their ages range 2-15year with mean of 7.2 ± 3.6 year.

Ethical considerations:

- The study purpose and procedures were explained to the parents and written consents were obtained before the study
- Approval of the local ethical committee in the pediatrics department and General Organization for Educational Institutes and Hospitals were obtained before the study
- The authors declared no potential conflict of interest with respect to the research &publication of this article.
- All data of the patient &results of the study are confidential and

the patient has the right to keep it

• The authors received no financial support for the research &publications of the article.

Inclusion criteria (for group 1&2):

children with acute dysentery, acute watery or foul-smelling diarrhea ,abdominal complaints as pain, repeated attacks of diarrhea flatulence, cramps and/ or bloating, malaise, fatigue , anorexia with or without manifestations of malnutrition.

Exclusion criteria:

Patients on antacids, laxative, antimicrobials and those have food allergy or metabolic disorders.

Those children were divided into two groups.

Group (1):

Included 40 children (26 males and 14 females) with acute dysentery or foul-smelling diarrhea their stool samples were examined for detection of E. histolytica

Group (2):

Included 40 children (21 males and 19 females) with abdominal complaints as pain, repeated attacks of diarrhea and/ or flatulence, malaise, fatigue, anorexia with or without manifestations of malnutrition their stool samples were examined for detection of G. lamblia.

Group (3):

Included 30 healthy children (16 males and 14 females) without any complaints with normal growth rate their stool samples were examined for detection of both E histolytica and G. lamblia

The studied groups were subjected to:

Complete history taking including age, sex, residence, complaints including diarrheal history: type, period, drug intake or chronic use of enema or suppositories, food allergy or metabolic disorders.

Complete thorough examination general and local for abdomen.

Laboratory evaluation by:

- Stool analysis by microscopic examination for trophozoites and/ or cysts of G. lamblia or E. histolytica
- Stool examination for coproantigen for E. histolytica and /or G. lamblia.
- Single stool specimen was collected in a clean container from each of the participant and was divided into two parts. One part for diagnosis by ELISA antigen detection and was

preserved at-20 ˚C until analysis while the other part for microscopy and was processed immediately.

Two types of direct wet film preparation were done for each fresh unpreserved stool sample at the same time. A small amount was suspended in a drop of normal saline on one slide and was covered with slip for detecting the actively motile trophozites. In a second microscope slide a drop of Lugol's iodine was added to the stool smear according to native Lugol examination for detecting the cysts and /or trophozites of E. histolytica or G. lamblia **(Cheesbrough,1998)** Both stool smears were examined microscopically at low (10x) and high (40) objective lenses.

The copro antigens for E. histolytica or G. lamblia were detected by using Ridascreen Entamoeba and Giardia kit made by R-Biopharm AG, Darmstadt, Germany **(Mannweiler 1995)**. The microtiter wells were coated by monoclonal antibodies specific for E. histolytica or G. lamblia. The stool samples were diluted 1:11 by sample diluent buffer. The diluted stool was added to well coat with enzyme conjugate and incubated for 60 min at room temperature the unbound conjugate is washed off. After addition of the substrate solution, the intensity of color developed was measured at wave length of 450 nm.

The results were obtained by using cut-off value which was determined by addition of 0.15 to the measured absorption of the negative control. Samples were considered positive if the extinction is more than 10% above the calculated cut-off

Statistical analysis:

Statistical comparisons were performed with the SPSS program for Windows (version 18.0, SPSS, Chicago, IL).

Since Ridascreen Entamoeba and Giardia ELISA test kits made by R-Biopharm AG were reported that it could identify pathogenic E. histolytica or G. lamblia only. It was nominated as a reference standard test

RESULTS

Table (1): Demographic characters of studied groups

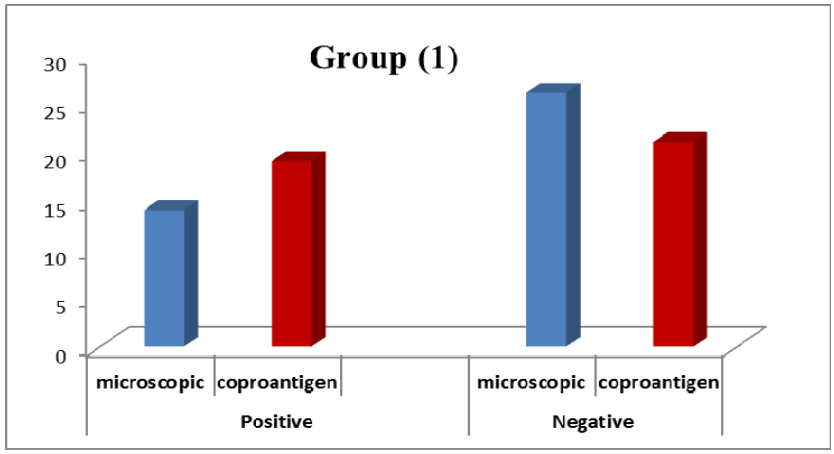
	Group (1) no=40	Group (2) no=40	Group (3) no=30
Age (years): Range	2-9	4-14	2-15
Maen ±SD	4.3± 2.36	9.2± 1.83	7.4± 3.72
Sex : Male	26 (65%)	21 (52.5%)	16 (53.3%)
Female	14 (35%)	19 (47.5%)	14 (46.7%)
Residence: Rural	29 (72.5%)	23 (57.5%)	14 (46.7%)
Urban	11 (27.5%)	17 (42.5%)	16 (53.3%)

Table (2): microscopic examination versus Coproantigen ELISA test for group (1)

Group (1) (for E. histolytica) n=40	positive	negative
Microscopic examination	14 (35%)	26(65%)
Coproantigen ELISA test	19(47.5%)	21(52.5%)
sensitivity	73%	
specificity	100%	
PPV	NA	
NPV	80.7%	

This table shows that examination of group (1) for E.histolytica: by microscopic examination 14 patients were positive (35%) and 26 were negative (65%) while by Coproantigen ELISA test 19cases were positive including the 15positive cases detected by microscopy (47.5%) and 21 case were negative (52.5%)

Figure (1): Microscopic examination versus Coproantigen ELISA test in group (1)



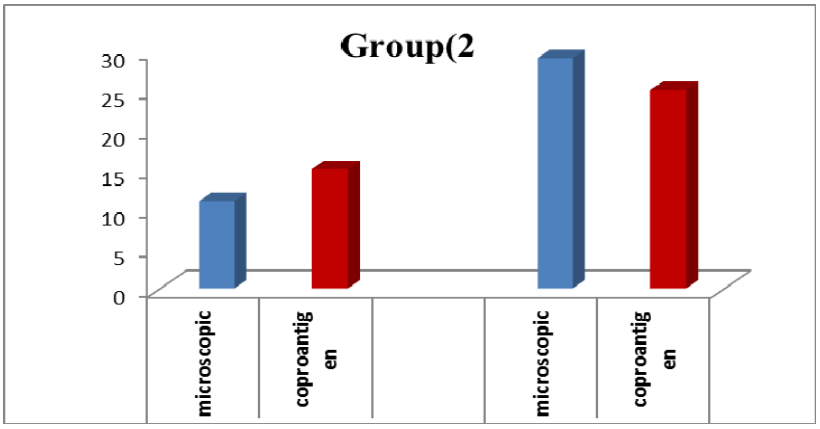
This figure shows that microscopic examination detects less positive cases and more negative cases than Coproantigen ELISA test.

Table (3): microscopic examination versus Coproantigen ELISA test for group (2)

Group (2) n=40 G. lamblia	positive	negative
Microscopic examination	11(27.5%)	29(72.5%)
Coproantigen ELISA test	15(37.5%)	25(62.5%)
sensitivity	73.3%	
specificity	100%	
PPV	100%	
NPV	86.2%	

This table shows that examination of group (2) for G. lamblia: by microscopic examination 11 patients were positive (27.5%) and 29 were negative (72.5%) while by Coproantigen ELISA test 15cases were positive including the 11microscopically positive cases (37.5%) and 25 case were negative (62.5%)

Figure (2): Microscopic examination versus Coproantigen ELISA test in group (2)



This figure shows that microscopic examination for Giardia detects less positive cases and more negative cases than Coproantigen for Giardia ELISA test.

Table (4): microscopic examination versus Coproantigen ELISA test for group (3)

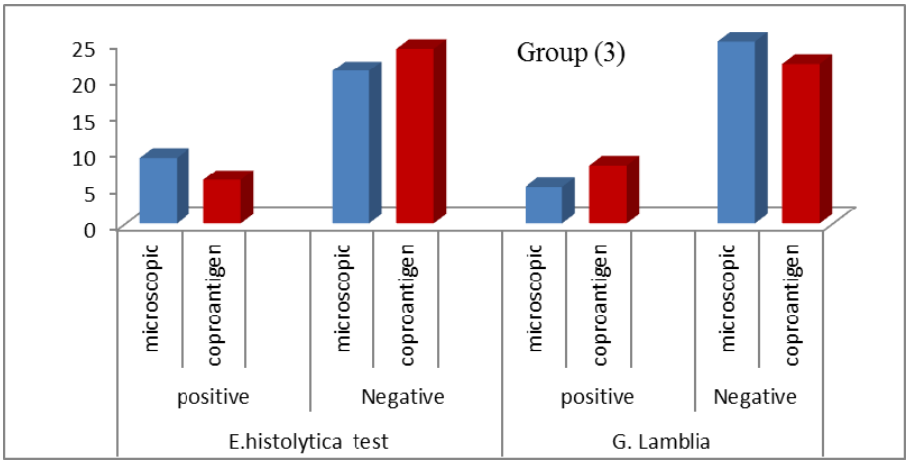
Group (3)n=30	E. histolytica		G.lamblia	
	positive	negative	positive	negative
Microscopic examination	9(30%)	21(70%)	5(16.6%)	25(83.4%)
Coproantigen ELISA test	7(23.3%)	23(76.7%)	8(26.6%)	22(73.4%)
Sensitivity	100 %		62.5%	
Specificity	91.6%;		100%	
PPV	77.7%		100%	
NPV	100%.		89.2%.	

This table shows that examination of group (3) for E.histolytica: by microscopic examination 9 patients were positive (30%) and 21 were negative (70%) for trophozoit or cysts while by Coproantigen ELISA, only 7 of the 9 microscopically positive cases were positive (23.3%) and 23 cases were negative (76.7%).

Also examination for Giardia lamblia :by microscopic examination 5patients were positive (16.6%) and 25 were negative (83.4%) while by Coproantigen ELISA all the 5 cases were positive in addition to 3 new cases i.e.

8positive cases (26.6%) and 22 case were negative (73.4%).

Figure (3): Microscopic examination versus Coproantigen ELISA test in group (3)



This figure shows that microscopic examination for E.histolytica detects more false positive cases and less true negative cases than Coproantigen for ELISA test.

It also shows that microscopic examination for Giardia detects less positive cases and more negative cases than Coproantigen ELISA test.

DISCUSSION

Diagnosis of E histolytica and/ or G. lamblia by stool microscopy is relatively inexpensive and approximately 85% of cases are detected when three separate stool samples are examined but in practice only a single stool exam is performed **(Saber et al., 2011** and **Shahat et al., 2017)**. Also, morphologic similarity between E hitolytica and E. dispar causes over estimation of E histolytica infection **(Van Den Broucke et al., 2018)**.

The current study compares the specific coproantigen level and microscopic examination as tools for diagnosis of G lamblia and E histolytica infections.

Examination of stool of group (1) (included 40 children with acute dysentery for detection of E. histolytica) by microscopic examination 14 patients were positive (35%) and 26 were negative (65%) while by Coproantigen ELISA test

19cases were positive including the 15 microscopically positive cases (47.5%) and 21 case were negative (52.5%) %) [Sensitivity 73%, specificity 100% and NPV 80.7%] this means that Coproantigen testing more senistive for detection of the presence of E. histolytica than microscopic examination in acute infective cases.

Examination of stool of group (2) with abdominal complaints, anorexia with or without manifestations of malnutrition for G. lamblia by microscopic examination 11 patients were positive (27.5%) and 29 were negative (72.5%) while by Coproantigen ELISA test 15 cases were positive including the 11 microscopically positive cases (37.5%) and 25 case were negative (62.5%)%) [Sensitivity 73.3%, specificity 100% PPV 100% and NPV 86.2%]. So,Coproantigen ELISA test more sensitive with higher NPV in patients with Giardia infection than microscopic examination.

Examination of stool of group (3) that included normal children without any complaints and with normal growth rate for detection of both E histolytica and G. lamblia as carriers.

For E. histolytica: by microscopic examination 9 patients were positive (30%) and 21 were negative (70%) while by Coproantigen ELISA test 7 only of the 9 positive cases were positive (23.3%) while the remaining 23 cases were negative [sensitivity 100% , specificity 91.6 % PPV 77.7% and NPV 100 %].

So Coproantigen ELISA test more specific with higher PPV than microscopic examination for carrier cases of E. histolytica, as it detect only E.histolytica and no other entamebae species that have similar morphology but different antigen.

As regards G. lamblia: by microscopic examination 5patients were positive (16.6%) and 25 were negative (83.4%) while by Coproantigen ELISA test 8positive cases (26.6%) and 22 cases were negative (73.4%). [Sensitivity 62.5%, specificity 100 % PPV 100% and NPV 89.2 %] .So, Coproantigen ELISA test is more sensitive with higher NPV than microscopic stool examination for carrier cases of G. Lamblia.

These results coincided with **(Tanyuksel et al., 2005)** and **(Leo et al., 2006)** who reported some advantages of ELISA kits

over direct microscopy as: high sensitivity and specificity, rapid technique, unneeded experienced personnel and absence of cross-reaction against other parasites.

Also **(Saber et al., 2011** and **Ibrahim et al., 2015)** who had reported that E. histolytica coproantigen ELISA detection surpassed its microscopical detection. Also, coproantigen is more reliable for specific detection of E histolytica infection than stool analysis as there is no cross reactivity with other types of Entameobae.

In the present study, the prevalence of Giardia was 27.5% by direct microscopy and 37.5% by ELISA in cases with abdominal complaints as diarrhea and /or flatulence, malaise, anorexia with or without manifestations of malnutrition. This is comparable to **(Noor et al.2014** and **Singhal et al.2015)**, studies where the prevalence rates of Giardia by direct microscopy were 15.5% and 17.3% respectively and by ELISA were 22.6% and 23.6% respectively this may be due to environmental sanitation differences.

(Jahan et al., 2014) had evaluated the efficacy of Giardia (ELISA) test and direct microscopy in the diagnosis of G. lamblia in stool specimens from patients with diarrhea and other gastrointestinal symptoms and found that (22.6%) were positive for G. lamblia. Maximum cases were detected by (ELISA) test with sensitivity of 100% and specificity of 91.5%.

On the other hand, **(Garcia et al., 2003)** and **(Selim et al., 2015)** stated that false negative results for Giardia with ELI SA were obtained when small numbers of parasites are present in stool and microscopic examination was taken as the gold standard in the diagnosis of giardiasis.

CONCLUSION

Coproantigen (ELISA) test for E. histolytica or G. lamblia is more reliable than microscopic stool analysis and is considered as a rapid and effective method with high sensitivity and specificity, so it is useful as a supplement of stool examination in survey studies and in outbreaks as it allows examination of large number of cases in short time thus reducing the chances of missing asymptomatic cases and avoid unnecessary treatment for other non-pathogenic species .

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مستضد الكوبرو لطفيلي الجارديا لامبليا اوالانتامييا هستوليتيكا في براز الاطفال كمقياس يعتمد عليه لتشخيص الاصابه بهما في مستشفي بنها التعليمي

د/ جيهان فريد عريبي – د/ عبير السيد حامد - د/ سحر حسين قشوه - د/ لبني ياسين موسي

تعتبر الاصابه (بالانتامييا هستولتيكا) و(الجارديا لامبليا) من اكثر الامراض انتشارا بين الاطفال. وقد تتسبب في الاصابه بالنزلات المعويه وسوء التغذيه. وللتشخيص عاده ما يستخدم الفحص الميكروسكوبي للبراز، ولكن لا تكون النتائج سليمه بدرجه كبيره حيث ان افراز الاكياس تكون غير منتظمه والطفيل النشط (التروفوزيت) يتحلل بسرعه فقد لا يظهر تحت الميكروسكوب. بالاضافه لعدم التفرقه بين انواع(الانتامييا) الغير مرضيه بالميكروسكوب فقط . ولذلك استحدث (مستضد الكوبرو) كوسيله مضمونه للتشخيص.

الهدف من البحث : تقييم مستوي مستضد الكوبرو في براز الاطفال المشتبه باصابتهم بالجارديا او الانتامييا هستوليتيكا كمقياس يعتمد عليه لتشخيص الاصابه بالطفيل في مستشفي بنها التعليمي.

وقد اجريت الدراسه علي مائه وعشره طفلا بعد اخذ موافقه اهلهم لعمل الدراسه قسموا الي ثلاث مجموعات:

المجموعه الاولي: تشمل اربعون مريضا(بدوسنتاريا) معويه تم فحص برازهم (لانتامييا هستولتيكا) بالميكروسكوب وباستخدام مستضد (الكوبرو للانتامييا).

المجموعه الثانيه: تشمل اربعون مريضا باعراض معويه مختلفه تم فحص برازهم (للجارديا لامبليا) بالميكروسكوب وباستخدام (مستضد الكوبرو للجارديا).

المجموعه الثالثه: تشمل ثلاثون طفلا سليما لايعانون من اعراض معويه وطبيعي النمو تم فحص برازهم (للانتاميبا هستولتيكا) و(للجارديا) بالميكروسكوب وباستخدام (مستضد الكوبرو للانتاميبا و للجارديا).

وقد تم جدولة النتائج ومقارنتها احصائيا:

و قد بينت الدراسه ان قياس مستوي (مستضد الكوبرو) لطفيلي (الجارديا لامبليا اوالانتاميبا هستوليتيكا) في براز الاطفال اكثر دقه وتخصص لهذين الطفيليين ومقياس يعتمد عليه لتشخيص الاصابه بهما وبالتالي سرعه علاجهما وتجنب مضاعفاتهما ويوصي بعمل دراسات اوسع علي فئات اكثرلتاكيد هذه النتائج.