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GIARDIA INTESTINALIS: EVALUATION OF ELISA COPROANTIGEN IN DIAGNOSIS AND EFFECT OF NITAZOXANIDE AND METRONIDAZOLE IN TREATMENT OF GIARDIASIS IN CHILDREN

By

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ABSTRACT

Background: Giardiasis is one of the most common intestinal parasitic infections causing diarrheal illness in humans worldwide. Detection of *Giardia intestinalis* is traditionally performed by microscopic examination of stool specimens. Nitroimidazoles group (metronidazole and tinidazole) are conventional drugs of choice for treatment of Giardiasis with a cure rate of higher than 90%. All of these drugs may lead to numerous adverse reactions, require long duration therapy, and none of them is absolutely safe for use during pregnancy.

Objective: This study was undertaken to evaluate the performance of the ELISA coproantigen for the diagnosis of Giardiasis and to compare the efficacy and safety of Nitazoxanide versus Metronidazole in the treatment of giardiasis.

Subjects and methods: A total of 350 children, aged 6-12 years, of both sexes were randomly selected for parasitological investigation using direct wet mount and formol-ether concentration techniques. The *Giardia* infected cases and 20 free of parasites were subjected to ELISA coproantigen test. Eighty cases infected with *Giardia intestinal is* were divided randomly into 2 equal groups: Group (1) were given nitazoxanide (200 mg twice daily for 3 days respectively), and Group (2) were given metronidazole (20 mg/kg thrice daily for 7 days). To evaluate the effectiveness of the therapy, at least three stool samples from all cases were examined after completion of the treatment. A standardized questionnaire was used to record Clinical symptoms of the patients in each group prior to and after treatment.

Results: The prevalence of giardiasis in our study was 23.7% . In our study, enzyme linked immunosorbent assay for coproantigenic detection of *G. intestinalis* has a sensitivity of 94.9% and a specificity of 85.7% with PPV of 92.5%, and a NPV of 90 %. The two treated groups were similar with respect to sex and mean age. The cure rate was 95% and 85% for Nitazoxanide and Metronidazole respectively with statistically significant difference.

Conclusion: The results of this study suggested that coproantigenic technique by ELISA test is suitable for use in testing a larger number of samples, especially for screening persons in regions where *G. intestinalis* is a common wide pathogen. Also, it confirms the efficacy and safety of nitazoxanide as a 3-day treatment of giardiasis in children.

INTRODUCTION

Intestinal parasitic infections are most common among school age children aged 5-15 years and were attributed to poor sanitation and hygiene. These infections can affect educational achievement, reproductive health, social and economic

developments (**Nematian et al., 2008)**. The prevalence of these infections and the extent of their public health effect in Egypt are not clearly understood. The flagellated protozoan *Giardia intestinalis* is one of the most common intestinal parasites affecting humans worldwide. It is estimated that 200 million people in the developing countries have symptomatic giardiasis (**Bilenko et al., 2004).**

The prevalence of infection varies widely depending on the sensitivity of the diagnostic method (**Flanagan, 1992)**. Giardiasis may be asymptomatic or responsible for a broad clinical spectrum, including acute or chronic diarrhea which may be with or without dehydration and malabsorption syndrome, nausea, vomiting, abdominal pain, flatulence and weight loss are also commonly reported (**Ortiz et al., 2001).**

Detection of *Giardia intestinalis* is traditionally performed by microscopic examination of stool specimens. Repeating this examination once or twice on additional specimens improves the sensitivity of the test due to the intermittency of cyst excretion **(Ortega & Adam, 1997 and Gupta et al., 2003).**

The sensitivity of microscopy is dependent on the skill of the microscopist and the time spent scanning each preparation. Efforts have been made to improve the sensitivity of the diagnosis of *Giardia*. Some of the methods have been investigated for automating the detection of *Giardia* species, including immuno-fluorescent assay, enzyme immunoassay, counter immunoelectrophoresis and radioimmune precipitation assay (**Garcia and Shimizu, 1997).**

The nitroimidazoles, metronidazole and tinidazole are conventional drugs of choice for treatment of giardiasis with a cure rate of higher than 90%. All of these drugs may lead to numerous adverse reactions, require long duration therapy and none of them is absolutely safe for use during pregnancy (**Dutta et al., 1994).**

Nitazoxanide, (2-acetyloloxy-N (5-nitro-2thiazolyl) benzamide), is the only agent that has broad coverage against both common intestinal parasitic protozoa and helminthes (**Ochoa and White, 2005).**

Nitazoxanide interferes with pyruvate ferredoxin-oxidoreductase (PFOR) enzyme dependent electron transfer reaction which is important for anaerobic glucose energy metabolism resulting in cell swelling, membrane damage and vacuole injury of the trophozoites, resulting in dysfunction of the parasite (**Abd el-Rahman et al., 1997).**

This study was undertaken to evaluate the performance of the RIDASCREEN® (R-Biopharm AG, Darmstadt, Germany) *Giardia* kit for the prevalence of giardiasis and to compare the efficacy and safety of Nitazoxanide versus drug of choice Metronidazole in the treatment of giardiasis.

MATERIALS AND METHODS

A randomized case- controlled trial study, was carried out at the Department of Parasitology at Al-Azhar University between the period from March 2015 to August 2015. A total of 350 children, aged 6-12 years, of both sexes were randomly selected for parasitological investigation. The children were examined for *Giardia* cysts and/or trophozoites using direct wet mount and formal-ether concentration techniques **(Smith and Paget, 2007).** Microscopic examination

consisted of two wet mount preparations for each fecal specimen; one non-stained and the other stained with iodine. Informed consent of their parents or themselves was provided.

Sample collection: Fresh stool specimens (5-10 grams) were collected in clean plastic containers, and examined within 24 hours from the disposal of feces (**Garcia, 2007).** Gross examination of the sample was performed for color, consistency, mucus, blood and adult parasites. The sample was then divided into two parts: From the first part, direct wet mounts and formal-ether concentration examinations were carried out. The second part was immediately stored at -20°C for performing ELISA of *G. intestinalis*. The *Giardia* infected cases were 34 males and 46 females, and mean age was 8.28±2.14 y (infected group), and 20 subjects were 12 males and 8 females, and mean age was 8.60±2.23y (healthy control group) were subjected to: ELISA coproantigen test using RIDASCREEN® ELISA test (r-Biopharm AG, Darmstadt, Germany) according to manufacturers method.

Eighty cases, aged 6-12 years infected with *Giardia intestinalis* were divided randomly into 2 equal groups:

Group (1): Cases were given nitazoxanide 200 mg twice daily for 3 days respectively.

Group (2): Cases were given metronidazole 20 mg/kg thrice daily for 7 days.

The criteria for inclusion were:

(1) Single infection with G. *intestinalis*. (2) Able to take oral medication. (3) not known to have contraindications to Nitazoxanide or Metronidazole (4) not received any anti-parasitic chemotherapy in the previous 2 months . Those who were not able to attend follow-up examinations were excluded from the study.

To evaluate the effectiveness of the therapy, at least three stool samples from all cases were examined on the 5th, 10th and 15th day after completion of the treatment. A standardized questionnaire was used to record clinical symptoms of the patients in each group prior to and after treatment.

Statistical analysis: The collected data were organized, tabulated and statistically analyzed using SPSS, version 18 (USA). Using direct microscopy as the gold standard test for diagnosis of giardiasis. RIDASCREEN ® *Giardia* ELISA kit was evaluated for sensitivity, specificity, positive predictive value, and negative predictive value. For quantitative data, the mean and standard deviation were calculated. The difference between two means was statistically analyzed using the students t- test. For qualitative data, the number and percent distribution was calculated. Chi (X^2) square were used for significance assossiation. The results of $P < 0.05$ were considered statistically significant.

RESULTS

The prevalence of giardiasis in the present work was (23.7%) (83/350). Enzyme linked immunosorbent assay for coproantigenic detection of *G. intestinalis* had a sensitivity of 94.9% and a specificity of 85.7% with PPV of 92.5% and a NPV of 90 % table (1). The two treatment groups were similar with respect to sex and mean age ($p>0.05$) table (2). There is non-significant difference

between treated groups as regard clinical manifestations abdominal pain, nausea, vomiting, constipation, distention and flatulence, steatorrhea and loss of appetite but it was significant as regard diarrhea. The children complained of more than one symptom and sign table (3). In the present study the cure rate was 95% and 85% for Nitazoxanide and Metronidazole respectively with statistically non significant difference ($p>0.05$) table (4).

Table (1): Efficiency of Elisa coproantigen using microscopy as a gold standered method.

Test	Sensitivity	Specificity	PPV	NPV
ELISA *Giardia* coproantigen	94.9%	85.7%	92.5%	90%

PPV: positive predictive value NPV: negative predictive value

Table (2): Personal data of treated groups.

Character \ Groups		Group (1) NO. = 40	Group (2) No. = 40	P-value
Age	Mean ±SD	8.75± 2.12	7.65 ± 2.03	0.12
	Range	6-12	6-12	
Gender	Male	18	16	0.65
	Female	22	24	

Table (3): Pre and post treatment clinical data in the treated groups .

Parameters \ Groups		Group (1) No. = 40		Group (2) No. = 40		P-value
		No.	%	No.	%	
Abdominal pain	Pre	32	80	28	70	0.43
	Post	4	10	6	15	
Nausea	Pre	16	40	20	50	0.21
	Post	0	0	2	5	
Vomiting	Pre	15	37.5	12	30	0.21
	Post	2	5	0	0	
Diarrhea	Pre	26	65	29	72.5	0.026
	Post	0	0	6	15	
Distention & flatulence	Pre	24	60	16	40	0.75
	Post	4	10	2	5	
Constipation	Pre	8	20	12	30	1.00
	Post	2	5	3	7.5	
Steatorrhea	Pre	22	55	18	45	0.13
	Post	0	0	2	5	
Loss of appetite	Pre	34	85	28	70	0.56
	post	5	12.5	6	15	

Table (4): Efficacy of nitazoxanide and metronidazole in treated groups.

Groups / Treatment effect	Group (1) NO. = 40		Group (2) No. = 40	
	No.	%	No.	%
Cure after at least one stool exam.	38	95	34	85
No cure	2	5	6	15
P-value	0.13			

DISCUSSION

Giardiasis is one of the most common intestinl parasitic infections causing diarrheal illness in humans worldwide. The infection rate is 2-7% in developed countries and 20-30% in developing countries **(Bilenko et al., 2004).** The prevalence of giardiasis in the present work was 23.7 % in accordance with that reported in other studies from Egypt which varied between 14.8 and 30.8 % **(El-Kadi et al., 2006 and Sabry et al., 2009).** Also, it was somewhat similar to the 24.7% recorded in the Behera Governorate (**Curtale et al., 1998)**, lower than 33% among a sample of Cairo residents **(Shukry et al., 1986**), and **(Elswaifi et al., 2014)** who recorded that the prevalence was 38 % in Dakahlia Governorate .

In the present study, enzyme linked immunosorbent assay for coproantigenic detection of *G. intestinalis* has a sensitivity of 94.9% and a specificity of 85.7% with PPV of 92.5% and a NPV of 90 %. It was quick and convenient method for screening tests. This was in agreement with **Selim, et al. (2009)** who reported that ELISA technique for detection of *Giardia* copro-antigen had a sensitivity of 97.3% and a specificity of 82.6% with PPV of 80.4% and a NPV of 97.7%.

It was comparable to studies performed by **Duque-Beltron et al. (2002) , Guimar?es & Sogayar (2002) and Ozekinci et al. (2005)** where the sensitivity of ELISA for *Giardia* was 100%, 96.4% and 82%, respectively, and the specificity was 95%, 80.8% and 39%, respectively. Of the 360 cases, 17.2% samples were positive for *Giardia* by direct microscopy and 23.6% were found to be positive by ELISA (sensitivity ~97%), but specificity was ~92% only (**Singhal et al., 2015).** Also, **Jahan et al. (2014)** detected that the sensitivity and specificity of ELISA test in comparison with direct wet mount microscopy was found to be 100% and 91.5% respectively. In another study sensitivity and specificity of ELISA test was found to be 76.4% and 100% respectively (**Al-Saeed and Issa, 2010).**

In the present work, ELISA had a high sensitivity (94.9%) but a comparatively low specificity (85.7%). It was a very good diagnostic test at finding the disease because it was sensitive, but because of its lower specificity, it can give positive results when the disease is not actually present. Accordingly, false positive cases can be present because it is not very specific. This may be due to some cross-reactions with other intestinal parasites and some past infection with

giardiasis. However, if the ELISA result is negative, we can be fairly certain that the patient does not have giardiasis.

A patient was only considered to be cured if no *Giardia* trophozoites or cysts could be found in any of the three post-treatment fecal specimens. The two treatment groups were similar with respect to sex and mean age. The cure rate reached 95% and 85% for nitazoxanide and metronidazole respectively with non statistically significant difference. The frequency of parasitological cure after the nitazoxanide was a little higher than that obtained with metronidazole, but the difference was not statistically significant **(Canete et al., 2010).**

These results were similar to the results of **Ortiz et al. (2001)** who made a randomized clinical study of nitazoxanide compared to metronidazole in the treatment of symptomatic giardiasis in children from Northern Peru. Also, **Ali et al. (2014)** reported that the proportions of children resolving diarrhea (had no parasites in their stool) in the nitazoxanide group was higher than metronidazole group in giardiasis. The parasitological cure after the nitazoxanide in the present study was 95% higher than the 80.4% reported by **Rodr?guez-Garc?a et al. (1999)** in Mexican children, but similar to the 94% reported by **Abaza et al. (1998)** in Egypt.

Sadjadi et al. (2001) treated *Giardia lambilia* infected cases (7-12 years old) either with 200 mg mebendazole three times a day for 5 days or metronidazole with a daily 15mg/kg for 7 days and reported cure rates of 86% and 90% for mebendazole and metronidazole, respectively. Cure rate was 60%, 57.1%, 42.1%, 52% for albendazole, nitazoxanide, nitazoxanide-albendazole combination and placebo respectively for giardiasis (**Speich et al., 2013)**. Both treatment schedules were well accepted and well tolerated, with only mild, transient and self-limited side-effects reported (**Escobedo et al., 2008)**.

Although metronidazole has been a common and effective treatment for giardiasis, it has some disadvantages, such as long duration of treatment, a multiple-dose regimen and frequent side effects, such as a metallic taste, nausea, vomiting, abdominal cramps, headache, anorexia and neurological side effects. All of these features may result in poor compliance in a significant number of patients, especially children (**Raether and Hanel, 2003).**

CONCLUSION

ELISA test for detection of *Giardia* coproantigen is an alternative diagnostic method for microscopy and the efficacy and safety of nitazoxanide as a 3-day treatment of giardiasis in children. Further studies are needed on a larger sample size using other molecular tests in order to get more accurate estimations.

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الجيارديا المعوية: تقييم تحديد الأنتيجينات في البراز بإستخدام إختبار الإليزا في التشخيص وتأثير نيتازوكسانيد وميترونيدازول في علاج مرض الجيارديا في الأطفال

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خلفية البحث: الجيارديا هو أحد الأمراض الطفيلية المعوية الأكثر شيوعا والتي تسبب مرض الإسهال لدى البشر في جميع أنحاء العالم ، ويتم الكشف عن الجيارديا المعوية تقليديا عن طريق الفحص المجهري لعينات البراز، وتعتمد حساسية الفحص على مهارة إختصاصي المجاهر، والوقت الذي يقضيه مسح كل عينة. وتعد مجموعة نيتروإميدازول (ميترونيدازول وتينيدازول)هي الأدوية التقليدية لمعالجة مرض الجيارديا ، لكن كل هذه الأدوية قد تؤدي إلى العديد من الأعراض الجانبية، ويتطلب العلاج مدة طويلة وليست آمنة تماما للإستخدام خلال فترة الحمل.

هدف الدراسة: تقييم تحديد الأنتيجينات في البراز بإستخدام إختبار الإليزا لتشخيص مرض الجيارديا ولمقارنة فعالية وسلامة نيتازوكسانيد مقابل ميترونيدازول في علاج مرض الجيارديا.

الأشخاص وطرق البحث: أجريت الدراسة على 350 طفل، تتراوح أعمارهم بين 6-12 سنة، وتم إختيارهم عشوائيا من الجنسين، وتم فحص عينات البراز بطريقتى اللطخة المباشرة والترسيب بالفورمالين والكحول. وتم أخذ 80 حالة مصابة بالجيارديا و20 حالة خالية من الطفيليات لتحديد الأنتيجينات في البراز بإستخدام الإليزا، وتم تقسيم الحالات المصابة (80 حالة) عشوائيا إلى مجموعتين متساويتين: المجموعة الأولى: أعطي كل شخص200مللي جرام نيتازوكسانيد مرتين يوميا لمدة 3 أيام متوالية، والمجموعة الثانية: أعطي كل شخص ميترونيدازول 20 مللي جرام / كجم ثلاث جرعات يوميا لمدة 7أيام لتقييم فعالية العلاج، وقد تم فحص ثلاثة عينات من البراز لجميع الحالات بعد الإنتهاء من العلاج، وتم إستخدام إستبيان موحد لتسجيل الأعراض السريرية للمرضى في كل مجموعة قبل وبعد العلاج.

النتائج: أثبتت الدراسة أن نسبة إنتشار مرض الجيارديا هو (23.7٪) ، وتحديد الأنتيجينات في البراز بإستخدام الإليزا لتشخيص الجيارديا المعوية لديه حساسية 94.9٪، وخصوصية 85.7٪، والقيمة التنبؤية الإيجابية 92.5٪، والقيمة التنبؤية السلبية 90٪. وكانت مجموعتي العلاج متماثلة فيما يتعلق بالجنس والسن وليس بينهما فروقاً ذات دلالة إحصائية .وفي دراستنا كانت نسبة الشفاء 95٪ و 85٪ للنيتازوكسانيد والميترونيدازول على التوالي مع فروق ليست ذات دلالة إحصائية .

الإستنتاج: تشير نتائج هذه الدراسة إلى أن التشخيص بواسطة إختبار الإليزا لتحديد الأنتيجينات في البراز مناسب للإستخدام في إختبار عدد كبير من العينات وخاصة لفحص الأشخاص في المناطق حيث الجيارديا المعوية طفيلا واسع الإنتشار، كما تؤكد الدراسة على فعالية وسلامة نيتازوكسانيد كعلاج لمدة 3 أيام للأطفال المصابين بالجيارديا المعوية .