A Study of the Prevalence of Intestinal parasites for patients with chronic renal failure on hemodialysis

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Abstract

Background & Aim: Intestinal parasites infections are an important cause of morbidity and mortality for immunodeficiency patients; including patients with chronic renal failure on hemodialysis.

The study aims to determine the most important intestinal parasites and their prevalence for patients with chronic renal failure on hemodialysis, as they are immunocompromised in comparison with a group of healthy people.

Materials & Methods: The study has been conducted on 150 patients (80 female,70 males) aged between 13 -79 years , divided into two groups; the first group is 75 patients (43 females,32 males) with chronic renal failure on hemodialysis for a period of no less than 6 months, twice a week. The second group is the control group with 75 participants (36 females,39 males) who are healthy people, from which stool samples were examined microscopically to determine the intestinal parasite and its prevalence and a comparison between the two groups was drawn.

Results: the result have shown:

The prevalence rate in the patient group was 64% compared to 17.3% in the control group (OR=8.47, P value=0.0003), the most common parasites in the patient group were: Blastocystis hominis 28%, Giardia 14.6%, Entamoeba histolytica 10.6%, Entamoeba coli 5.3%, Cryptosporidium 2.6%, Iodamoeba butschilii 1.3%, Chilomastix mesnilii 1.3%, while in the control group was: Blastocystis hominis 9.3%, Entamoeba histolytica 2.6%, Entamoeba coli 2.6%, Giardia 1.3%, Iodamoeba butschilii 1.3%.

Conclusions: the statistics study has shown a strong correlation between the presence of intestinal parasites and patients with chronic renal failure on hemodialysis, compared to healthy people. Therefore, we recommend a routine stool examination for the patients to investigate the presence of intestinal parasites and treat these parasistes.

Key words: Intestinal parasites, Blastocystis hominis, chronic renal failure, hemodialysis

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دراسة انتشار الطفيليات المعوية لدى مرضى القصور الكلوي المزمن الخاضعين للديال الدموي

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الملخص

خلفية البحث و هدفه: تعد الأخماج الطفيلية المعوية سبباً مهماً للمراضة، والوفيات لدى مرضى نقص المناعة بما في ذلك مرضى القصور الكلوي المزمن الخاضعين للديال الدموي. وتهدف الدراسة إلى تحديد أهم الطفيليات المعوية، ونسبة انتشارها عند مرضى القصور الكلوي المزمن الخاضعين للديال الدموي باعتبارهم ضعيفين مناعياً مقارنة مع مجموعة من الأشخاص الأصحاء.

مواد البحث و طرائقه: دُرِسَتْ عينة مؤلفةً من 150 مريض (80 أنثى و 70 ذكراً) ، وتراوحت أعمارهم بين 13 و79 عاماً مقسمين إلى مجموعتين متساويتين: الأولى مجموعة المرضى 75مريضاً (43 أنثى+32 ذكراً) ، وهم من مرضى القصور الكلوي المزمن الخاضعين للديال الدموي منذ فترة لا تقل عن 6 أشهر بمعدل جلستين أسبوعياً، و الثانية مجموعة الشاهد 75 مشاركاً (36أنثى+39ذكراً) من الأشخاص الأصحاء ؛ إذ فُحصت عينات البراز مجهرياً ؛ لتحديد نوع الطفيلي المعوي، و نسبة انتشاره ، و المقارنة بين المجموعتين.

النتائج: أظهرت النتائج أن نسبة الانتشار في مجموعة المرضى كانت 64% مقابل 17.3% في مجموعة الشاهد (OR=8.47 ، وقيمة P value=0.0003) ، وكانت الطفيليات الأكثر شيوعاً في مجموعة المرضى هي الأكياس الأريمية البشرية بنسبة 28% ، وتليها الجيارديا14.6% ، والمتحول الحال للنسج 10.6% ، والمتحول الكولوني5.3% ، والبوغيات الخفية 2.6% ، واليودية البوتشيلية 1.3% ، وشفوية السياط المنيلية1.3% ، بينما في مجموعة الشاهد كانت نسبة الأكياس الأريمية البشرية9.3% ، والمتحول الحال للنسج2.6% ، والمتحول الكولوني2.6% ، والجيارديا 1.3% ، واليودية البوتشيلية1.3%.

الاستنتاج: أظهرت الدراسة الإحصائية علاقة ارتباط قوية بين وجود الطفيليات المعوية، وبين مرضى القصور الكلوي المزمن الخاضعين للديال الدموي مقارنة مع مجموعة الشاهد؛ لذلك نوصي بإجراء فحص روتيني لهؤلاء المرضى للتحري عن وجود الطفيليات المعوية ومعالجتها.

الكلمات المفتاحية: الطفيليات المعوية، الأكياس الأريمية البشرية، القصور الكلوي المزمن، الديال الدموي.

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Introduction:

Intestinal parasites

They are Parasites found in the gastrointestinal tract of humans and animals. they can be found in different parts of the body, but they prefer the intestinal wall. Most of these parasites cause infection of the gastrointestinal tract (gastroenteritis) accompanied by a number of symptoms, such as diarrhea, vomiting and abdominal pain. Harrison's internal Medicine .2003.

The most prominent intestinal parasites on humans:

Blastocystis hominis:

It is a heterogeneous protozoan intestinal parasite belonging to Stramenopiles (Heterokonts). Stechmann A, Hamblin K, and Perez-Brocal V .2008; 18(8):580-5.

It is one of the most common isolated intestinal parasites, with affects humans worldwide. . El Safadi D et al.2014;14:164. it is an important cause of morbidity for immunocompromised people. Jimenez PA, Jaimes JE, and Ramirez JD. 2019;12:376.

The pathogenesis is still a matter of controversy, but a recent studies show that 50-80% of infected people would have symptoms (diarrhea, nausea, abdominal cramps and anal itching…)

There are a number of factors that affect the appearance of symptoms (age, immune status, genetic changes that affect cytokine production…). Toro Monjaraz, E.M., et al. 2018. 64(4): p. 279-283.

Diagnosis: a parasite is seen by examination the stool in the form of circular elements (cysts) of varying sizes 2-15 microns, very shiny, containing a central vacuole that does not take iodine with several peripheral nuclei. . Khademvatan, S, et al.2018. 11(1): p. 43-47.

Amoebozoa:

It is phylum of protists, which includes a wide group of protozoa, amoebae that move by internal cytoplasmic flow (pseudolegs). It includes:

Entamoeba histolytica

It is the only pathogenic kind of Entamoeba on humans. It causes Amoebiasis and it is found all over the world. Healthy people who carry Entamoeba increase the spread of the disease by excreting cysts with stool. Mucosal and bloody diarrhea with a sense of tenesmus is the main symptom of acute amoebiasis. Behnia, M., et al 2008. 46(3): p. 153-6

The parasite is seen in one of the following forms:

- Histolytica form: motile trophozoite, 20-40 microns, and erythrocyte-infecting.
- Pro-cyst form: motile trophozoite, 10-12 microns, and non-erythrocyte-infecting.
- Cystic form: non-motile resistant, 10-13 microns, It contains one nucleus, two nuclei, or four nuclei depending on its maturity and the cysts are shed with stool.

Diagnosis: microscopic examining of stool or smears obtained by colonoscopy or pus abscesses and investigating of antigens in stool emulsion by immunochromatography.

Investigating of antibodies by serological tests, where antibodies are formed against trophozoites antigens in tissue invasive disease. Kantor M et al.2018:4601- 420.

Entamoeba coli:

It is the largest Entamoeba on humans. It lives in the lumen of the colon in one of two forms: trophozoite or cystic. Saritha Pujari.2015.

Less common Entamoeba:

Dispar.e, Entamoeba hartmanni, Entamoeba polecki, Dientamoeba fragilis, Iodamoeba butschilli, and Endolimax nana. Dolabella S .2012,107-117.

Cryptosporidium:

They are animal Sporozoites that infect humans, cause human coccidioidomycosis, and its symptom is diarrhea in healthy people and immunocompromised (watery diarrhea without mucus or blood that may last for months in immunocompromised).

Infection occurs when people eat vegetables and fruits and drink water contaminated with oocytes that are shed with the dung of infected animals. Infection also occurs when contact happens between humans and infected animals. Wesolowska, M., et al.2016. 62(3): p. 239-241

The parasite is seen in human feces in the form of oval cells measuring 4-5 microns and containing four crescent-shaped Sporozoites inside.

Diagnosis is made by stool smear staining with the modified Ziehl–Neelsen method in which the oocytes appear red. Investigating oocyte antigens in stool by latex or immunochromatography and

modern techniques of PCR. Johnston, S.P., et al.2003. 41(2): p. 623-6.

Giardia intestinalis

It is a single-celled flagella and one of the most common intestinal protozoa in the world (280 million infections in 2013, 7% in developed countries and more than 30% in developing countries). It affects all age groups. It is one of the most important causes of non-viral diarrhea. Coffey CM et al.2020.

The parasite is seen in the small intestine in two forms:

- Trophozoite form: 10-15 microns, motile, and with axial symmetry.
- Cystic form: oval, 13*8 microns, with a smooth double shell, and has four cores inside. Cysts form a means of infection in humans when eating contaminated raw vegetables and fruits. Currie, S.L., et al. 2017. 145(14): p. 3007-3011.

Diagnosis is made by examining the stool, where the cysts are clearly seen, while trophozoites that can be searched for in the duodenal aspirate (duodenal intubation) are rarely seen, and by detecting Giardia antigens in stool emulsion by immunochromatography and ELISA. Beer, K.D, et al.2017. 64(9): p. 1244-1250.

Other intestinal flagellates that parasitize on humans include:

Chilomastix mesnili that parasitizes the large intestine and it has one of two forms: trophozoite or cystic, Retortamonas intestinalis, Enteromonas hominis, and Trichomonas intestinalis.

Chronic Renal Failure:

It is an irreversible deterioration in renal function that develops over a period of years. It initial symptom is a biochemical disturbance that subsequently causes loss of the excretory, metabolic and endocrine functions of the kidneys. Clinical signs and symptoms of renal failure, which are due to what is known as high blood urea, develop and when death is possible without renal replacement therapy, the condition is called end stage renal failure (ESRF). Rahman M , Mahboob S, and Michael C.1998; 158(16):1743-52.

Patients with ESRF usually undergo dialysis which is the process of purifying the blood from toxic substances in two ways:

- Peritoneal dialysis: which uses the peritoneal membrane in the abdominal cavity.
- Hemodialysis: where the patient's blood is taken out and passed through filters that purify it and then return it to the patient's body. The dialysis aims to remove waste and toxic substances from the body and compensate for the loss of kidney function. This is done regularly either by entering the patient to the hospital or by visiting dialysis units in hospitals and outpatient clinics. Misra M. 2005;9-30 .

Immune disorders in hemodialysis patients:

Hemodialysis patients are more susceptible to infections because chronic renal failure is one of the immune-weakening diseases, as it negatively affects the humoral and cellular immunity in the body. In addition, one of the most important reasons for weak immunity is urea toxicosis, which causes disorders in the immune system that lead to a chronic immune inflammatory state. Hemodialysis itself (repeated individual contact with dialysis tools) also can increase the severity of this condition, which in the long-term leads to immunodeficiency for hemodialysis patients. Therefore, infections are the second cause of death in hemodialysis patients after the cardiovascular diseases. Leou S, Garnier F, and Testevuide P 2013;9:137-42.

Search objective:

- Studying the prevalence of intestinal parasites in a group of chronic renal failure patients on hemodialysis (as they are immunocompromised patients) and comparing it with the prevalence of parasites in a group of healthy people.
- Determining the types of present intestinal parasites and comparing the percentage of their presence in both groups.

Materials and methods:

The study sample: Total sample size: 150 participants. Study mode: "case- control study", (P- value < 0.05, confidence interval 95%) the sample was divided into two equal groups:

- the first group (the patient group) : includes 75 patients (43 females,32 males) aged between 13 -79, patients with chronic renal failure on hemodialysis for a period of no less than 6 months, and examined twice a week.

Patients with hemodialysis less than 6 months ago and who had a disease or other immune-compromising cause were excluded from the study.

The second group (the control group) : includes 75 healthy people accompanying patients (36 females,39 males) aged between 13 -79. A creatinine analysis was performed for all participants to confirm the presence of normal renal function, and those who had any other immunosuppressive cause were excluded.

- The objective of the study was explained to the participants who met the inclusion criteria, and their consent was taken, while ensuring the confidentiality of the information.

Place and time of study:

The study was conducted between 2019-2021.

Samples were collected from the dialysis department at AL-Mouasat University Hospital.

Creatinie analysis was performed in the central laboratory of AL-Mouasat University Hospital; the device is "Hitachi,Olumbus".

The samples were examined microscopically microscopically in the microbiology laboratory at the Faculty of Medicine- Damascus University.

Methodology:

Stool samples were collected from patients in airtight plastic containers to prevent contamination and were transferred to the laboratory for:

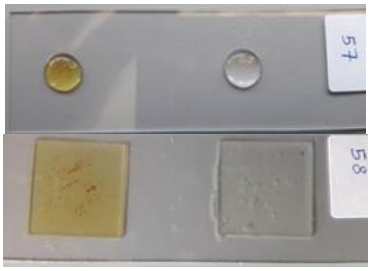
- Microscopic direct examination−
- Microscopic examination after addition of lugol−
- Fecal smear procedure and staining by the modified Ziehl–Neelsen method.

Microscopic examination:

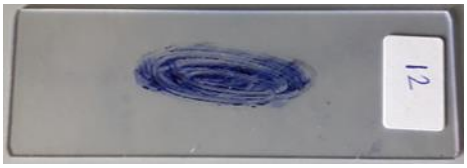
Microscopic direct examination:

After homogenizing the stool, a small amount is taken and emulsified in the physiological serum, and another amount is emulsified in a drop of iodine. the microscope is examined with magnification 10*then 40*.

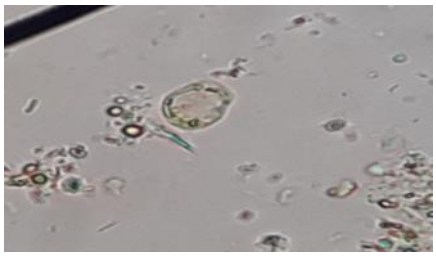
Detection of cryptosporidium: we prepare a fecal smear and stain it with the modified Ziehl–Neelsen method, which is the best method for diagnosis (carbol fuchsin and acid alcohol decolorize are used with methylene blue as a contrast color). the microscope is examined with magnification 40*then 100*(submersible lens).



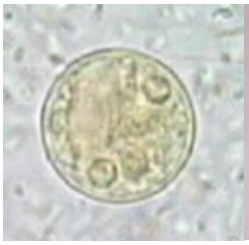
Figure(1): sample preparation for microscopy



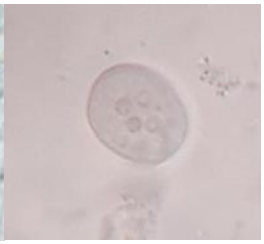
Figure(2): preparation of stool smear stained by modified Ziehl–Neelsen



Figure(3):blastocystis hominis wet stool (researcher's lens)



Figure(4): cystic form Entamoeba histolytica wet stool (researcher's lens)



Figure(5): cystic form Entamoeba coli wet stool (researcher's lens)

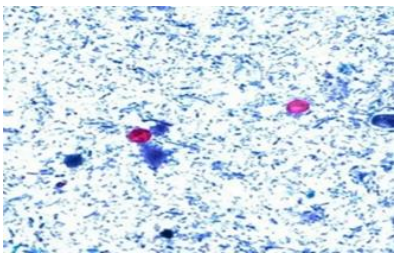


Figure (6): Cryptosporidium in stool smear stained by modified Ziehl–Neelsen (researcher's lens)

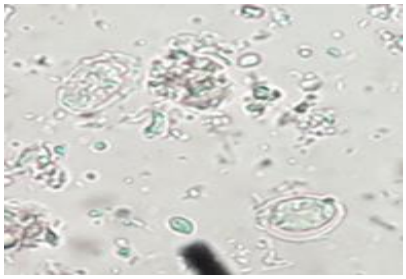


Figure (7): cystic form Giardia intestinalis wet stool (researcher's lens)

Statistical study:

The research sample consisted of 150 patients (79 female,71 males) aged between 13 -79 years divided into two groups: the patient group, and the control group.

Table (1) shows the distribution of patients in the research sample by gender.

Table(1): the distribution of patients in the research sample by gender

The studied group	Males	Females	Total number of patients	Percentage
the patient group	32	43	75	50%
the control group	39	36	75	50%
Total	71	79	150	100%

The sample was distributed into homogeneous age groups according to Sturgess law, according to the length of the group C = R /3.322logn + 1 where the category range is 10

Table (2) shows the distribution of patients in the research sample by age.

Table(2): the distribution of patients in the research sample by age

Age categories	13-22	23-32	33-42	43-52	53-62	63>	Total
the patient group	4	8	22	15	12	14	75
the control group	2	5	19	23	18	8	75

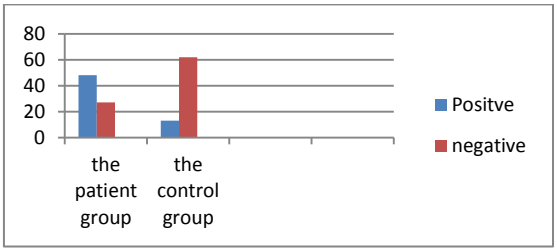
Statistical analysis and results:

After conducting a microscopic examination of the samples in both groups, the following was found:

the patient group: intestinal parasites were found in 48 samples(positive) compared to 27 negative samples that did not contain parasites.

the control group: intestinal parasites were found in 13 samples(positive) compared to 62 negative samples.

Chart(1):shows the distribution of positive and negative cases in the two study groups



Chart(1): the distribution of positive and negative cases in the two study groups

By calculating the prevalence of intestinal parasites in the two study groups, we find that the prevalence rate in the patients group was 64%, compared to 17.3% in the control group.

Table(3) : shows the prevalence of intestinal parasites in the two study groups.

Table(3) : the prevalence of intestinal parasites in the two study groups.

The study sample	Microscopy result		Total
	The presence of parasites	No parasites	
the patient group	64%	36%	100%
the control group	17.3%	82.7%	100%

- After determining the type of parasite present in the studied sample, it was found that: Table(4) : shows the prevalence of each parasite in the two study groups.

Table(4) : the prevalence of each parasite in the two study groups.

Parasite	the patient group		the control group	
	The number	The prevalence rate	The number	The prevalence rate
Blastocystis hominis	21	28%	7	9.3%
Giardia	11	14.6%	1	1.33%
Entamoeba histolytica	8	10.6%	2	2.6%
Entamoeba coli	4	5.3%	2	2.6%
Cryptosporidium	2	2.6%	0	0%
Iodamoeba butschilii	1	1.33%	1	1.33%
Chilomastix mesnilii	1	1.33%	0	0%
Total	48	64%	13	17.3%

- To study the difference between the percentages, a Z test was performed for the whole sample and for each parasite separately, and the P value was calculated.

Table(5): the application of the Z test to study the relationship between the percentages of parasite prevalence in the two study groups and for each parasite separately

Parasite	The prevalence rate		Z	P value	Decision
	the patient group	the control group			
Blastocystis hominis	28	9.3	3.02	0.0013	Significant
Giardia	14.6	1.33	3.1	0.0013	Significant
Entamoeba histolytica	10.6	2.6	1.99	0.023	Significant
Entamoeba coli	5.3	2.6	0.83	0.2709	Not significant
Cryptosporidium	2.6	0	*	*	*
Iodamoeba butschilii	1.33	1.33	0	1	*
Chilomastix mesnilii	1.33	0	*	*	*
Total	64	17.3	8.61	0.0002	Significant

*Significant at the level of significance 0.05

The prevalence of intestinal parasites in the group of patients was 64% compared to 17.3% in the control group, and the calculated Z value of the difference between the two percentages was 8.61, P value 0.0002 , it is a statistically significant value at a significance level less than 0.05 , which indicates a strong statistically significant relationship.

- To determine the likelihood of the presence of intestinal parasites in the group of patients, the odds ratio and risk factor were calculated for the whole sample and for each parasite separately.

Table(6) shows the odds ratio and risk factor values for the whole sample and for each parasite.

Table(6) : the odds ratio and risk factor values for the whole sample and for each parasite.

Parasite	Odds ratio (OD)	Risk factor	
		the patient group	the control group
Blastocystis hominis	3.77	3	0.79
Giardia	12.71	11	0.86
Entamoeba histolytica	4.35	4	0.91
Entamoeba coli	2.05	2	0.97
Cryptosporidium	5.13	0.97	0
Iodamoeba butschilii	1.0	1	1
Chilomastix mesnilii	3.04	0.98	0
Total	8.47	3.69	0.43

The odds ratio is 8.47, which is a statistically significant value, and the risk factor for the patient group was 3.69 and for the control group was 0.43 at the confidence level 95%.

It is clear that the most common intestinal parasite in both groups is Blastocystis hominis, where the prevalence rate in the patient group was 28% compared to 9.3% in the control group, and the calculated Z value of the difference between the two percentages was 3.02, P value 0.0013, it is a statistically significant value at a significance level less than 0.05 , which indicates a strong statistically significant relationship.

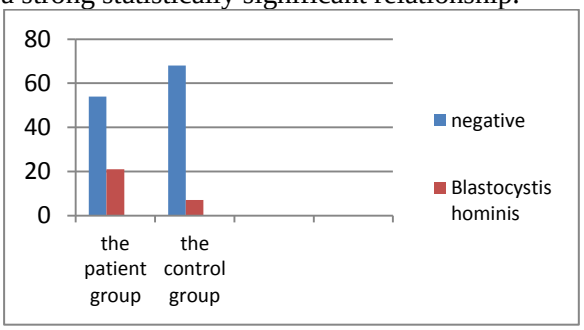


Chart (2) : the distribution of Blastocystis hominis in two study groups

Discussing the results and comparing them with international studies:

Intestinal parasites were found with a high prevalence of 64% in the hemodialysis patients group, compared to the control group(OR=8.47,Risk factor 3.69, P value 0.0003) it is a statistically significant difference at the confidence level 95% .

this indicates a strong correlation between chronic renal failure and hemodialysis as an immunocompromised cause and the presence of intestinal parasite in the stool.

Blastocystis hominis were the most common parasite in both groups, but it was found with a higher prevalence 28% in the hemodialysis patients group, compared to the control group (OR=3.77, Risk factor 3, P value 0.0013) which increases the possibility that Blastocystis hominis are an opportunistic parasite whose pathogenicity increases with the presence of an immune-compromising cause such as chronic renal failure and hemodialysis. Boer MD, Schuurs TA, and Vermeer M.2020;39:197.

several studies have been conducted to evaluate the effectiveness of treatment in symptomatic people with Blastocystis hominis, where the percentage of clinical improvement was as high as 79.5%. Lissette B.2017;40(6):381-387.

while the presence of cryptosporidium was not observed in the control group while it was found by 2.6% in the patient group- this is consistent with many international studies.

A study was conducted in Iran in 2017 on intestinal parasites infections in groups of immunocompromised patients, which included hemodialysis, kidney transplant, cancer, AIDS compared to healthy people, where the percentage of intestinal parasites in the dialysis patient was 11.9% compared to 0% in the control group, and blastocystis hominis was the most common 4.2% afflicting giardia 3% . Rasti S,and Scand j.2017.

A study was also conducted in Turkey in 2013 on intestinal parasites in hemodialysis patients compared to healthy people. intestinal parasites were found in a percentage of 43.7% in the patient group, while it was 12.7% in the control group, the most common parasites were

blastocystis hominis 23.9%, Giardia 8.5%, Entamoeba histolytica 2.1%, cryptosporidium 2.1%. Karadag G.2013.

A systemic review of meta- analysis was conducted on intestinal parasites in hemodialysis patients from developing countries 2020. Twenty- two studies included 11 using a case-control design and 11 studies using a cross-sectional design. cross sectional studies suggested that the prevalence was 24%. In studies using case- control design, the prevalence was 30% which was found to be significantly higher than controls. With regard to the parasites cryptosporidium and Blastocystis hominis. Ali Taghipour et al jan;24(1):12-21.2020

Conclusion:

intestinal parasites are more common for chronic renal failure on hemodialysis, as they are immunocompromised patients compared to healthy people. Blastocystis hominis are the most common of these parasites, followed by Giardia and Entamoeba histolytica, in addition to the presence of some parasites such as Cryptosporidium, which are more commonly found in immunocompromised.

Recommendations:

Routine stool examination for patients with chronic renal failure on hemodialysis to investigate the presence of intestinal parasites including Blastocystis hominis, that may be an important cause of gastrointestinal disorders and the resulting complications for these patients.

Treatment of positive symptomatic cases.

Applying preventive treatment in some cases to improving quality of life for patients.

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