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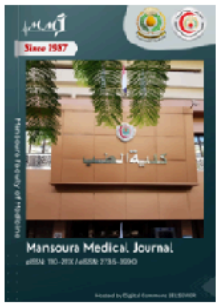

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INCIDENCE AND RISK FACTORS FOR CRYPTOSPORIDIUM INFECTION AMONG HEMODIALYSIS PATIENTS: RELATION TO SERUM IPTH LEVELS.

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By

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ABSTRACT

Background: Patients with chronic kidney disease are suffering from different electrolytes and hormonal changes. The effect of calcium, phosphorus and parathyroid hormone (PTH) levels as risk factors for *Cryptosporidium* infection have not been studied before.

Aim : To estimate the incidence of *Cryptosporidium* infection among hemodialysis patients and its relation to calcium, phosphorus and iPTH levels.

Patients and Methods : Seventy nine hemodialysis patients attending the hemodialysis Unit at Mansoura Urology and Nephrology Center and Mansoura University Hospital, and 80 apparently healthy controls were recruited in this study. All studied groups were subjected to history taking and clinical examination. Stool samples were examined macroscopically and microscopically and modified Kinyoun's acid fast stain for detection of intestinal coccidian was used. Blood samples were collected for estimation of iPTH, calcium, phosphorous, serum creatinine and plasma urea.

Results : among the 79 hemodialysis patients 49.4% had intestinal

parasites. The most prevalent parasite was Cryptosporidium (40.5%). The duration and efficiency of hemodialysis played a role in the increased incidence of parasitic infection (P= 0.031, 0.004 respectively), iPTH level played additional role (P=0.002). Efficiency of hemdialysis was the most predictor risk factor for intestinal parasitic infection among hemodialysis patients (OR =0.19, CI=0.05-0.82, P=0 0.02).

Conclusions : Cryptosporidium is a common parasitic infection in hemodialysis patients, special stain should routinely used in stool examination for its detection. Improving the efficiency of hemodialysis may help to reduce the incidence of parasitic infections. The iPTH is associated with increased incidence of Cryptosporidium infection and this needs further studies to confirm its role.

Keywords : Hemodialysis, Intestinal parasites, Cryptosporidium, Parathyroid hormone, Phosphorus, Calcium.

INTRODUCTION

Infection remains one of the major causes of morbidity and mortality among hemodialysis patients. Emerging intestinal parasites have gained increasing attention as important opportunistic pathogens responsible for clinically significant infections in immunocompromised patients.[1] Protozoal infections in such patients are common as cryptosporidiosis, isosporiasis, cyclosporiasis, and giardiasis.[2] *Strongyloides stercoralis* is a common helminth that cause severe infection among them.[3]

Secondary hyperparathyroidism and hyperphosphatemia are highly prevalent among end stage renal disease (ESRD) patients and have been studied as a possible factor in the development of infection among these patients.[4-8] However, the evidence about the role of intact parathyroid hormone (iPTH), calcium and phosphorus level in increasing the risk of parasitic infection among them is still conflicting.

Many studies showed increased prevalence of *Cryptosporidium* infection among maintenance hemodialysis patients [8,14,15,24]. The risk factors especially the electrolyte and parathyroid hormone levels that may predispose to this infection have not been studied before, so the aim

of this study is to demonstrate the incidence and risk factors that may predispose to intestinal parasitic infection especially C*ryptosporidium* among hemodialysis patients.

SUBJECTS AND METHODS

In this study, 79 hemodialysis patients undergoing maintenance hemodialysis at Urology and Nephrology Center and Mansoura University Hospital. In addition, 80 apparently healthy matched controls not complaining of any chronic diseases e.g. diabetes, liver diseases, malignanc, were recruited in the study. Exclusion criteria involved history of parathyroidectomy and having antiparasitic treatment in the previous six monthes. The protocol met the requirement of the local institutional ethics board and informed consents from all patients prior to the study were done. Participants underwent maintenance HD three times weekly using hollow-fiber dialyzers and bicarbonate dialysates containing calcium and magnesium at concentrations of 2.5 to 3.5 and 1.0 mEq/L, respectively.

All patients were subjected to history taking (age, gender, residence& duration of hemodialysis) and clinical examination.

Collection and examination of stool samples:

Stool samples were taken from patients in special plastic container and transferred to laboratory at the end of each working day. Stool samples were collected and examined macroscopically for consistency, color, odor, and the presence of blood, mucus, and gross parasites. Stool samples were divided into two parts: One part designated for direct smear and stool culture, direct wet smear was done for detection of helminths or protozoa. The second part was preserved in formalin to be examined by formalin-ether concentration method.[9]

Modified acid-fast stain: Smeared slides were prepared, air dried and fixed with absolute methanol for one min, then they were stained with carbol fuchsin for 5 min. After that slides were rinsed briefly (5 seconds) with 50% ethanol then thoroughly washed with water. The slides were decolorized with 1% sulfuric acid for 2 min. then rinsed with water and drained. Counter-stain, methylene blue was added to the

slides for 1 min. Finally the slides were rinsed with water and left to dry in air.[10]

Harada-Mori filter paper strip culture: for detection of S*trongyloides stercoralis* larvae.[11]

Blood sample collection and examination:

Pre-dialysis blood samples (5 ml) were collected under aseptic condition and the serum was separated. Biochemical testing for estimation of serum creatinine, complete blood count, calcium (reference range 8.8-10.4 mg/dl), phosphorous (reference range 2.5-4.3 mg/dl) and iPTH levels were done. Plasma urea level also was estimated. Urea reduction ratio (URR) was calculated to evaluate efficiency of hemodialysis (>65% considered efficient dialysis).[12] Detection of anti-Strongyloides stercoralis antibodies was done using an ELISA technique (DRG® Strongyloides IgG (EIA-4208) DRG International Inc., USA) for the qualitative screening of serum anti-Strongyloides IgG antibodies. The levels of iPTH were measured by the electro Chemi-Luminescence Immuno Assay (ECLIA) method using Roche Elecsys PTH kits (reference range 10-65 pg/ml).

STATISTICAL ANALYSIS

Data were analyzed using SPSS version 16 for windows (SPSS, Chicago, IL). Data were presented using mean ± standard deviation for all quantitative values, median for iPTH, and number of cases (percentage) for categorical variables. Normal distribution of continuous parameters was tested by Kolmogorov-Smirnov test. Categorical variables were analyzed using chi –square or fisher exact test whenever applicable. The significance of differences between continuous variables was determined with independent samples t- test or Mann-Whitney test whenever applicable. Logistic regression analysis was performed to identify independent predictors of intestinal parasitic infection. Statistical significance was determined as P values < 0.05.

RESULTS

The clinical and laboratory characteristics of the hemodialysis patients and controls were summerized in table (1). The mean duration of hemodialysis was 9.2±6.6 years. The studied groups were gender, age and residence matched (P > 0.05). The median iPTH level among hemodialysis patients was 495 pg/ml, (16.6-7689) (table 1).

Among the 79 hemodialysis patients, 39 (49.4%) had intestinal parasitic infection (Table 2) and mixed parasitic infection was detected in 34 (43%). Differences in the percentages of parasitism and mixed infection between the controls and the hemodialysis patients were significant (P<0.05 in both). The most prevalent parasites were *Cryptosporidium* (40.5%), B*lastocystis hominis* (27.8%) and *Entamoeba histolytica* (24.1%).

The median iPTH levels among patients with *Cryptosporidium* infection was significantly higher than those without *Cryptosporidium* infection (P=0.046) (table 3).

There were statistically significant difference between hemodialysis patients and controls as regard the phosphorus levels (P < 0.001), however there were no significant difference between patients with *Cryptosporidium* infection and those without (P>0.05)(table 3)

Risk factors for intestinal parasitic infections among hemodialysis patient were illustrated in table (4). Intestinal parasitic infection was significantly higher in patients with duration of hemodialysis > 5 years and URR < 65% (P =0.031 & 0.004 respectively).

The risk of infection with *Cryptosporidium* significantly increased with the duration of hemodialysis > 5 years, URR < 65% and iPTH level > 65 pg/ml (P =0.008, 0.03, 0.02 respectively) (table 5).

When doing multiple logistic regression analysis for risk factors, the URR is the only predictor for intestinal parasitic infection among hemodialysis patients (P= 0.03) (table 6). On the other hand none of these risk factors were predictor for Cryptosporidium infection among studied cases (P> 0.05).

Table 1: Demographic and laboratory characteristics of studied cases.

Variables	Patients N=79	Controls N=80	p
Gender (Male/Female)	(42/37)	(42/38)	0.9
Age (year)	48.8±15.1	48.1±12.6	0.8
Residence (Urban/Rural)	(40/39)	(45/34)	0.44
Hemoglobin (gm/dl)	9.8±1.9	13.4±1.1	**<0.001**
Platelet (count x 10^3)	187.5±79.3	220.4±68.7	**0.04**
Serum calcium (mg/dl)	8.8±0.9	9.4±0.3	0.06
Serum phosphorus (mg/dl)	5.4±1.5	3.3±0.5	**<0.001**
PTH (pg/ml)*	495(16.6-7689)	30.5(15-50)	**<0.001**

Values represent (means ± standard deviation), * represented (median (mximum-minimum)). ** represented (no (%)), N: number, PTH: parathyroid hormone, URR: urea reduction ratio.

Table 2: Frequencies of intestinal parasitic infections among studied cases:

Variable	Parasitized patients No (%)	Parasitized controls No (%)	p
Parasitic infection	39 (49.4%)	24(30%)	**0.015**
***Cryptosporidium* spp.**	32(40.5%)	6(7%)	**<0.001**
Blastocystis hominis	22(27.8%)	14(17.5%)	0.13
Giardia intestinalis	9 (11.4%)	0 (0%)	0.055
Entamoeba histolytica	22 (27.8%)	13(16.3%)	0.08
***Entamoeba coli*t**	8(10.1%)	0(0%)	0.176
Iodamoeba butschlii	3(3.8%)	0(0%)	0.553
***Enterobius vermicularis* egg**	2(2.5%)	0(0%)	0.9
Anti-*Strongyloides* antibodies	4(5.1%)	0(0%)	0.314
Mixed parasitism	34(43%)	2(2.5%)	**<0.001**

N, number

Table (3): Comparison between calcium, phosphorus and iPTH levels among hemodialysis patients with and without *Cryptosporidium* infections:

Variable	Patients		P
	Parasitized N=32	Non parasitized N=47	
Calcium (mg/dl)			
Mean± SD	8.9±	8.7±0.97	0.4
Phosphorus (mg/dl)			
Mean± SD	5.6±1.4	5.2±1.6	0.2
PTH (pg/ml)			
Median	630	297	
(minimum-maximum)	(98-3778)	(16.6-7689)	**0.046***

N, number ; PTH; parathyroid hormone, *, done by Mann-Whitney test.

Table 4: Risk factors for intestinal parasitic infections among hemodialysis patient.

Variable	Patients		p
	Parasitized N=39	Non parasitized N=40	
Age			
>50 year	23	20	
<50 year	19	17	0.654
Gender			
Male	22	20	
Female	17	20	0.654
Residence			
Urban	18	22	
Rural	21	18	0.503
Duration of HD			
<5 years	8	18	**0.031**
>5 years	31	22	
Efficiency of HD			
URR <65%	15	4	**0.004**
URR >65%	24	36	
Phosphorus			
<2.5 mg/dl	0	1	
2.5-4.3 mg/dl	5	13	0.06
>4.3 mg/dl	34	26	
Calcium			
<8.8 mg/dl	12	18	
8.8-10.4 mg/dl	26	19	0.194
>10.4 mg/dl	1	3	
PTH			
10-65 pg/ml	6	1	
>65 pg/ml	34	38	0.056
Leukocytic count			
<4000	8	6	0.211
4000-11000	30	23	
>11000	1	2	

N, number ; HD,hemodialysis, PTH;parathyroid hormone.

Table (5): Risk factors of *Cryptosporidium* infections among heamodialysis patient:

Variable	Patients		P
	Parasitized N=32	Non parasitized N=47	
Age			
>50 (years)	17	26	
<50 (years)	15	21	0.9
Gender			
Male	18	24	0.819
Female	14	23	
Residence			
Urban	12	28	0.069
Rural	20	19	
Duration of HD			
<5 years	5	21	
>5 years	27	26	**0.008**
Efficacy of HD			
URR <65%	12	7	**0.03**
URR >65%	20	40	
Phosphorus			
<2.5 mg/dl	0	1	
2.5-4.3 mg/dl	4	14	0.13
>4.3 mg/dl	28	38	
Calcium			
<8.8 mg/dl	9	21	
8.8-10.4 mg/dl	22	23	0.21
>10.4 mg/dl	1	3	
PTH			
10-65 pg/ml	0	8	
>65 pg/ml	32	39	**0.02**
Leukocytic			
<4000	7	7	
4000-11000	24	38	0.7
>11000	1	2	

N, number ; HD, hemodialysis, URR; urea reduction ratio, PTH;parathyroid hormone.

Table 6: Multiple logistic regression analysis for risk factors of intestinal parasitic among hemodialysi

Variable	Odds ratio	95% CI	p
Gender	0.73	0.25-2.11	0.6
Age (years)	1.70	0.32-1.59	0.4
Duration of dialysis (years)	2,62	0.8-8.57	0.1
Residence	1.12	0.38-3.29	0.8
URR (%)	0.19	0.05-0.82	**0.03**
Phosphorus	2.83	0.87-9.2	0.08
Calcium	1.43	0.52-3.91	0.5
Parathyroid hormone	7.66	0,73-80.01	0.08
Leucocytic count	1.23	0.34-4.54	0.8

CI, confidence inte

DISCUSSION

Intestinal parasitic infections is one of the major health problems among immunosuppressed patients, however, there is little information about them among hemodialysis patients.13-15 However, the evidence about the role of intact parathyroid hormone (iPTH), calcium and phosphorus level in increasing the risk of parasitic infection among them is still conflicting, so the aim of this work is to estimate the incidence of *Cryptosporidium* infections among hemodialysis patients and its relation to calcium phosphorus and iPTH levels.

In the present work, intestinal parasitic infections were detected in 49.4% of hemodialysis patients and in 30% of controls. Parasitism and polyparasitism were significantly higher in hemodialysis patients than controls. These results were in consistence with Kulik et al.16 On the other hand, our results exceeded the incidence of other studies.[17-18]

The increased incidence of infection may be related to profound immune dysfunction in ESRD patients. [19-21] Immunosuppress patients are very prone to develop enteric protozoan parasitic infection due to profound cell-mediated defects.[22] Also, hemodialysis patients may acquire infections through contact with nursing staff, equipments and materials.[23]

In our study, *Cryptosporidium* infection is the most prevalent intestinal parasite among hemodialysis patients. The same results were obtained by Baqai et al.[24] The same results also, were obtained but with lower prevalence in some studies.[8,14,15, 25]The low incidence of *Cryptosporidium* in other study,[18] could be attributed to none performing of a concentration method before staining. The high incidence of *Cryptosporidium* infection among our hemodialysis patients could be attributed to the fact that cellular immunity plays the major role against Cryptosporidium infection.[26]

The median iPTH levels were significantly higher among hemodialysis patients infected with *Cryptosporidium,* that may be due to the acquired T-lymphocyte dysfunction associated with hyperparathyroidism.[5, 27]

Evaluation of the risk factors for parasitic infections among parasi-

tized hemodialysis patients revealed that, the increased duration and low efficiency of the hemodialysis played a role in the increased incidence of infection. This agreed with some studies[17,28] and disagreed with the others.[15,25] This may be related to hemodialysis treatment per se, including dialysis membrane type and dialysate purity, which appear to play a significant role in the aggravation of the uraemia induced immune-dysfunction. [29, 30]

In our study, increased duration, decreased efficiency of hemodialysis, and hyperparathyroidism were associated with higher incidence of Cryptosporidium Infection.

In our work, the phosphorus levels is higher among hemodialysis patients (P<0.001) and parasitic infections were more frequent among patients with high phosphate levels although it does not reach significant level (P= 0.06). This result enforced the study produced by Plantinga et al.8 who reported that infections of any type were more frequent among patients with high phosphate level. Hyperphosphataemia was directly associated with diminished populations of naïve and central memory T lymphocytes and this may, in part, contribute to the acquired impaired immune response of ESRD.[31] Also phosphate may act purely as a surrogate for the uraemic state, which has also been associated with immune dysfunction.[8,32,33]

Efficiency of hemodialysis was the most predictor risk factor for intestinal parasitic infection among our hemodialysis patients (OR= 0.19, CI= 0.05-0.82) and this may be related to improvement of immune dysfunction after efficient hemodialysis.

The 4 positive cases for anti-*Strongyloides* antibodies by ELISA were negative by stool culture. Serologic response to *S. stercoralis* is known to cross-react with other parasites and this cross-reactivity resulted in false positive test.[34]

This study is limited by the no assessment of immune system and its relation to *Cryptosporidium* infection among hemodialysis patients.

In conclusion, *Cryptosporidium* is common parasitic infection in hemodialysis patients, special stain should be included as a routine test in stool examination for its detection. Among

hemodialysis patients, improving the efficiency of hemodialysis may help to reduce the incidence of parasitic infections. The iPTH is associated with increased incidence of *Cryptosporidium* infection among them and this need further studies to confirm its role.

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No conflict of interest.

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الملخص العربى

نسبة الاصابة وعوامل الخطر للاصابة بالكربتوسبوريديم فى مرضى الغسيل الكلوى: وعلاقتها بنسبة هورمون الغدة الجار-درقية فى الدم.

المرضى المصابون بامراض الكلى المزمنة يعانون ايضا من اختلالات فى نسبة الشوارد وتغييرات فى الهرمونات بالجسم. لم تتم من قبل دراسة تاثير تغير نسبة الكالسيوم والفسفور وهرمون الغدة الجار-درقية كعوامل خطر على نسبة الاصابة بالكربتوسبوريديم

الهدف من هذه الدراسة معرفة معدل الاصابة بالكربتوسبوريديم بين مرضى الغسيل الكلوى وعلاقتها بنسبة الكلسيوم والفسفور و هرمون الغدة الجار-درقية.

شملت هذه الدراسة 97مريض غسيل كلوى و 80 شخص سليم كمجموعة ضابطة. بعد عمل الفحص الاكلينيكى تم فحص عينات البراز وتم استخدام صبغة كينيون المعدلة للكشف عن الكوكسيديا المعوية. تم تجميع عينات دم من المشاركين لتحديد نسبة الكلسيوم والفسفور و هرمون الغدة الجار-درقية ونسبة الكرياتينين واليوريا فى الدم.

وكانت الاصابة بالكربتوسبوريديم هى الاعلى بين باقى الامراض الطفيلية فى الامعاء .(40.5%) وقد اثبتت النتائج ان مدة وكفاءة الغسيل الكلوى و نسبة هرمون الغدة الجار-درقية كان لهم دورذو دلالة احصائية فى زيادة الاصابة بالكربتوسبوريديم .

ونستنتج من هذا البحث ان الاصابة بالكربتوسبوريديم تنتشر بين مرضى الغسيل الكلوى ولكنها تحتاج الى استخدام صبغة خاصة لتشخيصها. وان تحسين نوعية الغسيل الكلوى يمكن ان يكون له دور فى تقليل نسبة الاصابة بهذا المرض الطفيلي. كما ان العلاقة بين هرمون الغدة الجار-درقية وزيادة نسبة الاصابة بالكربتوسبوريديم تحتاج الى المزيد من الدراسة.