EVALUATION OF INTESTINAL PARASITIC INFECTIONS IN CHRONIC KIDNEY DISEASE PATIENTS ADMITTED TO TERTIARY CARE HOSPITAL IN SOUTH ODISHA

Sibanarayan Jali[1], Pradeep Kumar Padhi[2], Deepak Kumar Naik[3], M. Nageswar[4], Diptimayee Tripathy[5]

[1]Assistant Professor, Department of General Medicine, MKCG Medical College, Berhampur, Ganjam, Odisha.
[2]Asssistant Professor, Department of General Medicine, MKCG Medical College, Berhampur, Ganjam, Odisha.
[3]Resident, Department of General Medicine, MKCG Medical College, Berhampur, Ganjam, Odisha.
[4]Assistant Professor, Department of General Medicine, MKCG Medical College, Berhampur, Ganjam, Odisha.
[5]Professor, Department of General Medicine, MKCG Medical College, Berhampur, Ganjam, Odisha.

ABSTRACT

BACKGROUND

There is paucity of data on prevalence of parasitic infections in patients of chronic kidney disease and in those undergoing haemodialysis. So, the present study was carried out with an aim to evaluate the prevalence of intestinal parasitic infections in patients of chronic kidney disease and those undergoing hemodialysis, and to correlate the prevalence of different parasitic infections in relation to severity of renal impairment in Chronic Kidney Disease (CKD) patients.

MATERIALS AND METHODS

This study was performed on 142 subjects suffering from Chronic Kidney Disease (CKD), defined as abnormalities of kidney structure or function, present for >3 months, with the implications for health, who satisfied the inclusion and exclusion criteria. The subjects in the study group (n=142) were divided into G3, G4, G5 category according to KDIGO, the number of subjects in each category being 33, 35, 74 respectively. G5 category subjects, for the purpose of the present study, were further subdivided into patients who were managed conservatively G5a (n=36) and patients who were undergoing haemodialysis G5b (n=38). The control group consisted of 30 healthy persons. Stool sample were collected in sterile, screw capped, plastic container from each patient and immediately transported to department of Microbiology. Jaffe's alkaline picrate method was used for estimating serum creatinine which is based on the principle that creatinine gives red colour of picramic acid with alkaline solution of picric acid. Kinetic UV assay method was used for estimating blood urea. Examination of stool consisted of macroscopic and microscopic examination.

RESULTS

A total of 172 subjects of both sexes and >18 years of age were included in the present study. Of these, 30 healthy volunteers were included in the control group and 142 patients with different severity of CKD (according to KDIGO criteria) including 38 patients on haemodialysis were included in the study group.

The subjects in the study group (n=142) were divided into G3, G4 and G5 groups according the KDIGO criteria. For the purpose of present study, the patients in G5 group were further classified into to two subgroups: patients on conservative management (G5a subgroup) and those on haemodialysis (G5b subgroup). Intestinal parasites were found in 21 (14.79%) of subjects in study group and 7(23.33%) in control group.

The prevalence of intestinal parasites was more in higher categories of CKD, 1(3.03%), 4(11.43%) and 16(21.62%) in CKD categories G3, G4 and G5 respectively. The difference in the prevalence of intestinal parasitic infections in different categories of CKD was statistically significant (p=0.036).

The prevalence of parasite was more in G5 as compared to G3 and G4 taken together, and the difference was statistically significant. (p=0.036).

Intestinal parasites were found in 12 (31.5%) subjects in G5 category who were undergoing haemodialysis (G5b) as compared to 9(8.65%) subjects who were not undergoing haemodialysis (G3+G4+G5a). The difference was statistically significant (p=0.002).

CONCLUSION

Prevalence of intestinal parasitic infections is higher in advanced chronic kidney disease patients and those undergoing haemodialysis. Hookworm infections were found to be predominant parasitic infections in chronic kidney disease patients in the present study.

KEYWORDS

Chronic Kidney Disease (CKD), Haemodialysis, Glomerular Filtration Rate (GFR), End Stage Kidney Disease (ESKD), Kidney Disease Improving Global Outcomes (KDIGO) CKD Work Group.

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Corresponding Author:
Dr. Pradeep Kumar Padhi,
Assistant Professor,
Department of General Medicine,
MKCG Medical College, Berhampur,
Ganjam, Odisha, India.
E-mail: drpkpadhy1973@gmail.com
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BACKGROUND

Chronic kidney disease (CKD) encompasses a spectrum of different pathophysiologic process associated with abnormal kidney function and a progressive decline in glomerular filtration rate (GFR). CKD is defined as abnormalities of kidney structure or function, present for >3 months with implications for health and is classified based on cause, GFR category and albuminuria category.[1]

Current CKD nomenclature used by 2012 KDIGO [1]

GFR Categories (ml/min/1.73 m ^{2})

G1: Normal or high	≥ 90
G2: Mildly decreased	60-89
G3a: Mildly to moderately decreased	45-59
G3b: Moderately to severely decrease	30-44
G4: Severely decreased	15-29
G5: Kidney failure	<15

Albuminuria Categories

A1: Normal to mildly increased <3 mg/mmol
A2: Moderately increased 3-30 mg/mmol
A3: Severely increased >30 mg/mmol

Prevalence of CKD is continuously increasing along with hypertension and diabetes.[2] End stage kidney disease (ESKD) can be defined by the requirement for life saving dialysis or kidney transplantation CKD causes progressive and irreversible loss of renal function resulting in accumulation of non-excreted metabolites by the kidney, such as urea,[3] which leads to uraemia and induces a state of immunosuppression[4,5] The CKD has negative impacts on neutrophil chemotaxis, phagocytosis, T cell functions and their bactericidal action also. Vitamin D deficiency in CRF also lead to diminished immune functions.[6] Haemodialysis treatment induces complement activation and release of pro-inflammatory cytokines and erythropoietin given to prevent anaemia among haemodialysis patients have been reported to have negative immune effects.[7,8] The immunosuppressed hosts are more likely to acquire infection after exposure, severe disease leading to dissemination once the infection is established rather than localized infection, the host is also unable to clear infections leading to chronic carriage states. These all account for the greater morbidity and mortality in these patients.[9] This state of immunosuppression (IS) attracts infections in general mainly bacterial, but intestinal parasitosis is also reported to be a clinically important infection in haemodialysis and renal transplant patients.[10] Worldwide very few studies have determined the prevalence of intestinal parasitic infections in patients of CKD and in those undergoing haemodialysis. The data in Indian set up is lacking and hence this study was undertaken.

Aim of the Study

To evaluate the prevalence of intestinal parasitic infections in patients of chronic kidney disease and those undergoing haemodialysis and to correlate the prevalence of different parasitic infections in relation to severity of renal impairment in Chronic kidney disease (CKD) patients.

MATERIALS AND METHODS

The present study is a Case control descriptive observational study, carried out in Department of Medicine MKCG Medical College, Berhampur, Odisha from April 2015 to March 2017. The study subjects were 142 patients (CASES) suffering from Chronic Kidney Disease (CKD), defined as abnormalities of kidney structure or function, present for >3 months, with the implications for health[1], who satisfied the inclusion and exclusion criteria. The cases in the study group (n=142) were divided into G3, G4, G5 category according to KDIGO, the number of subjects in each category being 33, 35, 74 respectively. G5 category subjects, for the purpose of the present study, were further subdivided into patients who were managed conservatively G5a (n=36) and patients who were undergoing haemodialysis G5b (n=38). The control group consisted of 30 healthy persons**.** (CONTROLS)

Inclusion Criteria

Adult patients (>18 years of age) of both sexes with established chronic kidney disease fulfilling KDIGO criteria of CKD category 3 to 5[1]

Exclusion Criteria

1. Patients suffering from any immunosuppressive disorders.
2. Patients taking any immunosuppressive medications.
3. Present or recent (preceding 3 months) use of antibiotics.
4. Patients undergoing peritoneal dialysis.

Study Methodology

Stool sample were collected in sterile, screw capped, plastic container from each patient and immediately transported to department of Microbiology.

Study Instruments

Jaffe's alkaline picrate method was used for estimating serum creatinine which is based on the principle that creatinine gives red colour of picramic acid with alkaline solution of picric acid.

Kinetic UV assay method was used for estimating blood urea.

Methods of Stool Examination[11]

Stool specimen were examined within half an hour of passage in order to maximize the chances for observing

motile trophozoites and prevent destruction of parasites due to delay.

Examination of stool consisted of macroscopic and microscopic examination.

Macroscopic Examination was done for consistency, colour and presence of blood, mucus or worms.

Microscopic Examination Included

1. Direct smear or film is made by mixing a small quantity of stool with a drop of liquid (e.g. saline, iodine) and examining first under low power (10X) then under high power (40X) of the microscope.

   Saline wet mount is made by mixing a small quantity of faeces with a drop of physiological saline. It is used to demonstrate helminthic eggs and larvae. It is also used to detect motile trophozoites of the intestinal protozoa.

   Iodine wet mount is made by using a drop of Lugol's iodine and mixing a small quantity of the stool sample and used for detection of protozoal cysts.

   Iodine stained cysts show pale refractile nuclei, yellowish cytoplasm and brown glycogen material. The motility of trophozoites is inhibited in the iodine wet mount.

2. Smear after Concentration

   Microscopy after concentration methods – when parasites were scanty in stools, routine microscopic examination may not give positive result. It then become necessary to concentrate the protozoan cyst and helminthic eggs and larvae by various methods. Several concentration techniques are in use which can be classified as salt floatation method and sedimentation method. In salt floatation method parasitic eggs and cysts floats in a solution of high specific gravity whereas they get sedimented in a solution of low specific gravity in sedimentation method of concentration.

   We used formalin ether sedimentation method and direct smears made from these concentrated samples were used to inspect for ova and cysts.

The procedure consists of the following steps:

A) Approximately 2 gm sample is taken in a 15 ml test tube. Mixed with 5-10% formalin and is allowed to stand for 30 minutes.
B) Sample is filtered through 2 layers of gauze piece to a 15 mi tube. Saline is added to the tube to bring the fluid level with several mm of the rim of the tube and is centrifuged for 10 min. at 500g.
C) The supernatant is discarded. The sediment is resuspended in 7 ml of 10% formalin & 3 ml of ether. Tube is closed with stopper and shaken vigorously for 30 seconds.
D) Tube centrifuged for 10 min at 500g and allowed to stand.
E) 4 layers are discovered. The bottom layer contains parasites, 2nd layer formalin, 3rd layer faecal debris and 4th layer of ether.
F) The layer of faecal debris is removed by an applicator stick. All liquid is discarded. The sediment is used for wet mount preparation as described earlier.

3. Permanent stained smears were used to confirm identification of certain intestinal protozoa and cysts.

   ❖ Kinyon acid fast stain (cold method)- procedure:

   a) Faecal smear is made on glass slide, air dried and fixed by adding methyl alcohol for 2 minutes.
   b) The slide is flooded with carbol fuschin for 5 minutes and then washed with water.
   c) Decolourising solution 1% sulphuric acid was kept for 2 min.
   d) Counter stained with methylene blue 0.3% for 1 min and slide then washed. Non-acid fast background stains blue with methylene blue. With high power this stain is used for identification of Cryptosporidium, Isospora and Cyclospora.

RESULTS

A total of 172 subjects of both sex and >18 years of age were included in the present study. Of these, 30 healthy volunteers were included in the control group and 142 patients with different severity of CKD (according to KDIGO criteria) including 38 patients on haemodialysis were included in the study group.

The subjects in the study group (n=142) were divided into G3, G4 and G5 groups according the KDIGO criteria. For the purpose of present study, the patients in G5 group were further classified into to two subgroups: patients on conservative management (G5a subgroup) and those on haemodialysis (G5b subgroup).

The number of male patients in the study group was more (79) than female patients (63), but the difference was not statistically significant. Similarly, there was no statistically significant difference in gender distribution between subjects in study and control group (Table 1). Intestinal parasites were found in 21 (14.79%) of subjects in study group and 7(23.33%) in control group, the difference was not statistically significant (p=0.278) (Table-4). The prevalence of intestinal parasites was more in higher categories of CKD, 1(3.03%), 4(11.43%) and 16(21.62%) in CKD categories G3, G4 and G5 respectively. The difference in the prevalence of intestinal parasitic infections in different categories of CKD was statistically significant (p=0.036). The prevalence of parasite was more in G5 as compared to G3 and G4 taken together, and the difference was statistically significant. (p=0.036). (Table-6). Intestinal parasites were found in 12 (31.5%) subjects in G5 category who were undergoing haemodialysis (G5b) as compared to 9(8.65%) subjects who were not undergoing haemodialysis (G3+G4+G5a). The difference was statistically significant (p=0.002) (Table-8). Intestinal parasites were found in 12 (31.5%) subjects in G5b category who were undergoing haemodialysis as compared to 5(7.35%) belonging to combined categories G3 and G4. The difference in the two groups was statistically significant (p=0.002) (Table-9)

Intestinal parasites were foung in 4 (11.11%) subjects in G5 category who were not undergoing haemodialysis as compared to 5(7.35%) belonging to combined categories G3 and G4. The difference in the two groups was not statistically significant (p=0.716) (Table-10) Intestinal parasites were found in 4 (11.11%) subjects in G5 category who were not undergoing haemodialysis (G5a) as compared to 12(31.58%) subjects in G5 category who were undergoing haemodialysis (G5b). The difference in the two groups was statistically significant (p=0.048) (Table-11).

DISCUSSION

The present study was undertaken to evaluate the prevalence of intestinal parasitic infestations in CKD patient and to find correlation, if any, between the prevalence of intestinal parasitic infection and the severity of CKD as defined by KDIGO criteria. The study also aimed at comparing the prevalence of intestinal parasites in patients on haemodialysis and patients of CKD, who were not on haemodialysis. The study included 30 healthy individuals who served as control group and 142 patients belonging to different categories of CKD as the study group. 142 patients in the study group included 38 patients who were undergoing haemodialysis.

The youngest patient in our study was 15 years and oldest 80 years old; the mean age being 44.7±14.38. The majority of patients belonged to the age group of 40 to 50 years. The mean age of controls was 42.07±10.04 years. There was no statistically significant difference in the age group in the study and the control group (p=0.234).

The number of male and female patients in the study group was 79 (59.63%) and 63 (44.36%) respectively. The control group had an equal number (50) of male and female patients. There was no statistically significant difference in the ratio of males and females between the control and the study groups (p=0.687). Amongst the study group (n=142), 33 (23.24%), 35 (24.65%) and 74 (52.11%) of patients belonged to G3, G4 and G5 categories CKD respectively (Table-2). Further among the G5 category, for the purpose of present study, patients were classified into two sub-groups: patients not on haemodialysis (G5a) and patients undergoing haemodialysis (G5b) and the number of patients in the subgroups was 36 and 38 respectively (Table-3). Intestinal parasites were found in 21 (14.79%) patients in the study group and in 7 (23.33%) subjects in the control group. However, the difference was not statistically significant between the two groups (p=0.278). The prevalence of intestinal parasites in general population has been found to vary considerably. The lowest prevalence has been reported by Turkupar et al[12] (0%) and highest by Dudeja M, et al[13] (26.1%). In our study the prevalence was found to be 23.3% and is thus almost similar to that observed by Dudeja M et al,[13] Kulik RA et al[14] (25.7%). In the present study, the prevalence of intestinal parasites increased with the increasing severity of CKD. The number of patients with intestinal parasites was observed to be 1 (3.03%), 4 (11.43%) and 16 (21.62%) in CKD categories G3, G4 and G5 respectively. (Table-6) The difference in the prevalence in different categories of CKD was statistically significant (p=0.036). There are no studies in literature which have examined the prevalence of intestinal parasites in different categories of CKD. In our study intestinal parasites were found in 12 (31.5%) subjects in G5 category who were undergoing haemodialysis (G5b) as compared to 9 (8.65%) subjects who were not undergoing haemodialysis (G5a). The difference was statistically significant (p=0.002). (Table-8)). The prevalence of intestinal parasites in patients undergoing haemodialysis has been found to vary between 3.8% and 51.6% as reported by Tappeh, et al[15] and Gil et al[16] respectively. The prevalence of intestinal parasites in patients undergoing haemodialysis in our study (31.5%) fall between that observed by Filho et al,[17] Tappeh et al,[15] Chief PP et al[18] and Turkucapar N et al[12] showing prevalence of 8.2%, 3.88%, 25% and 20.2% respectively which is lower than that in our study and the results of studies by Gil et al,[16] Kulik RA et al[14] and Seyrafian et al[19] showing prevalence as 51.6%, 45.1% and 43.9% respectively, which is higher than that in our study. Similar to results of our study, Karadeg et al.[20] Kulik RA et al[14] also found higher prevalence of intestinal parasites in haemodialysis as compared to subjects in the control group.

SEX	Groups		Total	P value
	CKD	CONTROL		
F	63 (44.37%)	15(50.00%)	78 (45.35%)	0.687
M	79 (55.63%)	15(50.00%)	94 (54.65%)	
Total	142 (100.00%)	30(100.00%)	172(100.00%)	

Table 1. Distribution of Subjects According to Gender

CKD CAT	Frequency	Percentage
G3	33	23.24%
G4	35	24.65%
G5	74	52.11%
Total	142	100.00%

Table 2. Distribution of Subjects in Study Group According to GFR

Undergoing Hemodialysis	Frequency	Percentage
No (G3+G4+G5a)	104	73.24%
Yes (G5b)	38	26.76%
Total	142	100.00%

Table 3. Distribution of Subjects into Subgroups on Conservative Management and Haemodialysis

Intestinal parasites	Group		Total	P value
	CKD	CONTROL		
Absent	121 (85.21%)	23 (76.67%)	144(83.72%)	0.278
Present	21 (14.79)	7 (23.33%)	28(16.28%)	
Total	142(100.00%)	30(100.00%)	172(100.00%)	

Table 4. Prevalence of Intestinal Parasites in Study Group and Control Group

SME	Group		Total	P value
	CKD	CONTROL		
B	2 (9.52%)	2 (28.57%)	4 (14.29%)	
C	2 (9.52%)	1 (14.29%)	3 (10.71%)	
E	4 (19.05%)	2 (28.57%)	6 (21.43%)	
G	3 (14.29%)	0 (0.00%)	3 (10.71%)	0.470
H	5 (23.81%)	0 (0.00%)	5 (17.86%)	
I	3 (14.29%)	2 (28.57%)	5 (17.86%)	
T	2 (9.52%)	0 (0.00%)	2 (7.14%)	
Total	21(100.00%)	7(100.00%)	28(100.005)	

Table 5. Prevalence of Intestinal Parasites in CD and Control Groups

B= Blastocystis Hominis,
C= Cryptosporidium Parvum
E= Entamoeba Histolytica
G= Giardia Lamblia
H= Hook Worm
I= Isospora Belli
T= Trichuris Trichura

Intestinal parasites	CKD CAT			Total	P value
	G3	G4	G5		
Absent	32(96.97%)	31(88.57%)	58(78.38%)	121(85.21%)	
Present	1(3.03%)	4(11.43%)	16(21.62%)	21(14.79%)	0.036
Total	33(100.00%)	35(100.00%)	74(100.00%)	142(100.00%)	

Table 6. Prevalence of Intestinal Parasites according to CKD Categories

SME	CKD CAT			Total	P value
	G3	G4	G5		
B	0(0.00%)	0(0.00%)	2(12.50%)	2(9.52%)	
C	0(0.00%)	0(0.00%)	2(12.50%)	2(9.52%)	
E	0(0.00%)	1(25.00%)	3(18.75%)	4(19.05%)	
G	0(0.00%)	1(25.00%)	2(12.50%)	3(14.29%)	0.868
H	1(100.00%)	1(25.00%)	3(18.75%)	5(23.81%)	
I	0(0.00%)	0(0.00%)	3(18.75%)	3(14.29%)	
T	0(0.00%)	1(25.00%)	1(6.25%)	2(9.52%)	
Total	1(100.00%)	4(100.00%)	16(100.00%)	21(100.00%)	

Table 7. Prevalence of Specific Intestinal Parasites in Different CKD Categories

Intestinal parasites	Hemodialysis		Total	P value
	No(G3+G4+G5a)	Yes (G5b)		
Absent	95(91.35%)	26(68.42%)	121(85.21%)	
Present	9(8.65%)	12(31.58%)	21(14.79%)	0.002
Total	104(100.00%)	38(100.00%)	142(100.00%)	

Table 8. Distribution of Intestinal Parasites in Subjects Belonging to G5a and G5b Sub-Groups of G5 Category

Absent	63(92.65%)	26(68.42%)	89(83.96%)	
Present	5(7.35%)	12(31.58%)	17(16.04%)	0.002
Total	68(100.00%)	38(100.00%)	106(100.00%)	

Table 9. Distribution of Intestinal Parasites in Group G3 and G4 Combined and G5b Subject Undergoing Haemodialysis.

Intestinal parasites	Group		Total	P value
	G3+G4	G5a		
Absent	63(92.65%)	32(88.89%)	95(91.35%)	
Present	5(7.35%)	4(11.11%)	9(8.65%)	0.716
Total	68(100.00%)	36(100.00%)	104(100.00%)	

Table 10. Distribution of Intestinal Parasites in Group G3 and G4 Combined and G5a Subject not Undergoing Haemodialysis

Absent	32(88.89%)	26(68.42%)	58(78.38%)	
Present	4(11.11%)	12(31.58%)	16(21.62%)	0.048
Total	36(100.00%)	38(100.00%)	74(100.00%)	

Table 11. Distribution of Intestinal Parasites between G5 Subgroups

CONCLUSION

Prevalence of intestinal parasitic infections is higher in advanced chronic kidney disease patients and those undergoing haemodialysis. Hookworm infections were found to be predominant parasitic infections in chronic kidney disease patients in the present study.

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