ORIGINAL RESEARCH PAPER
Volume - 10 | Issue - 02 | February - 2021 | PRINT ISSN No. 2277 - 8179 | DOI : 10.36106/ijsr
INTERNATIONAL JOURNAL OF SCIENTIFIC RESEARCH

A STUDY ON FUNGAL AND PARASITIC INFECTIONS AND ITS ANTIFUNGAL SUSCEPTIBILITY PATTERN IN PATIENTS ON HEMODIALYSIS IN A TERTIARY CARE HOSPITAL

Microbiology

Dr. R. Synthia Sevakumari* Assistant Professor of Microbiology, Government Medical College & ESI Hospital, Coimbatore, Tamil Nadu. *Corresponding Author

Dr. R. Deepa Associate Professor of Microbiology, Government Villupuram Medical College, Villupuram, Tamil Nadu.

Dr. G. Jeyalakshmi Former Director and Professor of Microbiology, Institute of Microbiology, Madras Medical College & RGGGH, Chennai, Tamil Nadu.

ABSTRACT

Patients undergoing Hemodialysis in Renal failure cases are at a higher risk of developing pathogenic and opportunistic fungal infections and parasitic infections due to the defective immune system. Accumulation of non excreted metabolites by kidney and associated chronic renal insufficiency leads to uremia and induces a state of immunosuppression that results in higher frequency of acquiring fungal and parasitic infection through Central venous catheters (CVC), contact with nursing personale, equipments, material on surface and from hands. Opportunistic intestinal parasitic infections are the most important cause of severe diarrhea in immunocompromised individuals, especially in patients undergoing hemodialysis. This cross sectional study was undertaken to determine the incidence of fungal and parasitic infection in Acute and Chronic Renal failure patients on hemodialysis with Central Venous Catheter, to analyze the associated risk factors and to perform antifungal susceptibility pattern. This study was done in the Institute of Microbiology in association with Department of Nephrology, Madras Medical College, Rajiv Gandhi Government General Hospital, and Chennai for a period of one year from September 2011- October 2012 **Materials and Methods:** A total number of 150 inpatients between the age group of 18-65 who are admitted for Hemodialysis in Nephrology ward were taken for the study. The risk factors associated in developing fungal and parasitic infections were observed. Fungal isolates from Blood, Catheter tip, swab from infected site, urine, Sputum were cultured by fungal culture and isolation of parasites in stool sample was done according to standard microbiological techniques. Antifungal susceptibility testing was done using standard CLSI guidelines.**Results:** A total of 5.6% of C.albicans, C.tropicalis, C.parapsilosis was isolated from blood. Aspergillus flavus was isolated only from catheter in 11.1% of cases and Aspergillus fumigatus in 5.6% of cases. Among the 4 cases Candida species isolated from catheter, 3 cases had candidemia and was treated with intravenous antifungal drugs. Out of 150 patients 13 patients had gastero intestinal symtoms Entamoeba histolytica was predominantly seen in 46.1% of patients with gasterointestinal symptoms followed by Giardia 30.7% , Cryptosporidium parvum 15.3% and Microsporidia 7.6%

KEYWORDS

Hemodiaysis, Immunosuppression, Slidecuture, Antifungal Susceptibility

INTRODUCTION

Invasive fungal infections have gained importance recently with increased incidence in patients with renal disease and kidney transplant recipients under effects of immunosuppression and environmental exposure [1,3,4]. Since they are frequently subtle in presentation and difficult to manage, early diagnosis and prompt therapy requires a high degree of suspicion and vigilance. Both cellular and humoral immunities are impaired and with the degree of impairment related to the duration of the uremic state which results in acquiring fungal and parasitic infections [2]. *Aspergillus flavus* and *Aspergillus fumigatus, C.albicans, C.tropicalis, C.parapsilosis* are the common fungal isolates seen in association with renal disease patients [5, 6, 7].

Opportunistic intestinal parasites such as *Cryptosporidium*, *Microsporidia*, *Isospora belli*, and *Strongyloides stercoralis* are the most important causative agents of severe diarrhea in immunocompromised individuals, especially in patients undergoing hemodialysis [1-3, 14, 15, 16]**.** In an immunosuppressed individual parasitic infection can cause profuse diarrhea, anorexia, malabsorption syndrome and weight loss which further worsens the general condition of the patient [17].

Although the prevalence of helminthic infections was very low among hemodialysis patients, [22- 25] one should not neglect these infections as they can still cause hyperinfections in immunocompromised persons [19, 20]. The occurrence of fungal, parasitic infection depends on the predisposing factors like long term use of immunosuppressive agents, broad-spectrum antibiotics, indwelling catheters, number of surgical procedures, associated co morbid conditions like Diabetes mellitus, Systemic hypertension, chronic anaemia and chronic liver disease [23,24]. The high rate of fungal infections in our patients is most probably related to the unhygienic conditions that continue to be prevalent in the tropical environment of third world countries [25].

The United States Centers for Disease Control (CDC) National nosocomial infection survey indicates that 82% of all episodes of nosocomial infections occurring in the hospitals are due to presence of long term CVC in a immunosuppressed patients is the single biggest risk factor for among HD patients [1].

All these infections are reversible and in view of the significant morbidity and mortality among patients on hemodialysis with CVC, early diagnosis, management and prevention fungal and parasitic infection is mandatory.

MATERIALS:

Blood, Catheter tip, swab from infected site, Sputum, Urine and Stool.

PROCESSING OF SPECIMEN:

Gram Staining

Thin smear of the specimen was prepared on a clean sterile glass slide and fixed by heating. The smear was flooded with 1% methyl violet for 1 minute and washed with distilled water and flooded with gram's iodine for 1 minute and washed with distilled water and decolorized with acetone, washed with distilled water and counter stained with dilute carbol fuschin for 30 seconds.

Wet Mount

In a clean glass slide about 2 mg of stool sample was mixed with a drop of saline, cover with a coverslip and examined under low and high power for trophozoites and cysts.

Iodine mount

In a clean glass slide small quantity about 2mg of stool was mixed with a drop of Lugol's iodine, cover with a coverslip and examined under low and high power for eggs.

KOH Wet Mount

A clean glass slide was taken. The specimen was placed in the centre of the slide. A drop of 10% KOH was added and a coverslip was placed over that, observed under low and high power microscope.

Giemsa Staining

Air dry and fix the smear with absolute methanol for 2-3 minutes. Stain the slide with Giemsa stain for 30 minutes. Air dry the slide and

observe under the oil immersion microscope.

Ziehl Neelsen staining
Smear was prepared on a clean glass slide and allowed to dry in the air and fixed by heating. The smear was flooded with strong carbol fuschin stain for 5-8 minutes with intermittent heating without boiling. The smear decolourized with 20% sulfuric acid and counterstained with loeffler's methylene blue for 1-2 minutes.

Modified Acid Fast Staining
The smear was flooded with strong carbol fuschin stain for 5 minutes. Washed with distilled water and flooded with 1% sulphuric acid for 3 minutes. Washed with distilled water and counter stained with 3% methylene blue for 3 minutes. Washed with distilled water dried and examined under oil immersion microscope.

Interpretation of Fungal Culture
Inoculated SDA slants were incubated at 25^0C and 35^0C for minimum of 4 weeks before discarding as negative. These slants were inspected daily during the first week and twice weekly during the next three weeks for growth. Identification of filamentous fungi was done by preparing Lacto Phenol Cotton Blue mount and studying the morphology of hyphae and conidial arrangement. In case of yeasts, identification and specification was done by Gram's stain morphology, germ tube test, Chrome agar and biochemical tests by standard microbiological techniques as recommended by CLSI.

Examination of Sabouraud's dextrose agar Media:
The colonies were observed for growth in the Sabouraud's dextrose agar slopes and noted the description, if it was inadequate reincubated and examined daily during first week and twice a week for next 3 weeks. Failure of growth after 6 weeks was considered as negative for fungal growth and is to be discarded.

Germ tube test (Reynolds-Braude Phenomenon)
Suspension of test organism in 0.5ml of sterile serum (plasma, egg albumin or peptone media) and incubated at 37^0C for 2 hours. A drop from incubated serum placed on the slide with coverslip and observed under microscope for germ tubes

Lactophenol cotton blue stain
The fungal growth was taken from Sabouraud's dextrose agar slope with spud and transferred onto the clean glass slide and two to three drops of Lactophenol cotton blue reagent was added over the fungal growth. By using teasing needles the growth was spread over the slide and coverslip was placed without trapping any air bubbles. The morphology of hyphae, conidia were observed under microscope and was correlated with macroscopic features.

Chrom Agar
The species of *candida* can be identified by different coloured colonies produced due to the reaction between specific enzymes of the different species and the chromogenic substrates in the system, which are as follows:

- *C.albicans* - light green
- *C.dubliniensis* - dark green
- *C.glabrata* - pink to purple
- *C.krusei* - pink
- *C.parapsilosis* - cream to pale pink
- *C.tropicalis* - blue with pink halo

Sugar Fermentation:
Biochemical tests like sugar fermentation was done for identification of yeast isolate Glucose, Maltose, sucrose, Lactose, Galactose and Trehalose sugars (2%) were used. The colour change in the tube containing the particular sugar indicates the yeast's ability to ferment carbohydrate.

Slide culture
The slide culture was performed using isolates. The slide culture is used to study undisturbed morphology details particularly relationship between reproductive structure like conidia, conidiophores and hyphae. Fungal slide culture was performed in cases with doubtful morphology.

ANTIFUNGAL SUSCEPTIBILITY TESTS
As per the guidelines of CLSI, the test was performed. MIC of water soluble drug Fluconazole and water insoluble drugs Amphotericin-B, Itraconazole and Voriconazole were determined.

BROTH DILUTION METHOD
Preparation of stock solution
Weight (mg) = volume (ml) x concentration (g/ml)/Assay Potency

Inoculum preparation
The isolates were subcultured on SDA, fresh culture of 24 hours colonies were taken 0.5 Mc Farland standard inoculum was prepared and diluted upto 1:20 with normal saline. The final inoculum was prepared. The Suspension was mixed for 15 minutes for turbidity adjusted ether visually or with a spectro photometer by adding sufficient sterile saline (or) adding more colonies and to match with 0.5 Mc Farland at 530 nm wave length with the optical density of 0.09 to 0.11.

Media used- RPMI 1640. Varying concentrations of the drugs were tested.

Amphotericin B	0.0313 to 16g/ml
Fluconazole	0.125 to 64g/ml
Itraconazole and Voriconazole	0.0313 to 16g/ml

Preparation
Dimethyl sulfoxide (DMSO) was used to dissolve Amphotercin B, Itraconazole and voriconazole. The DMSO, the series of dilution at 100 times the final concentration were prepared from the Antifungal stock solution.

ATCC *Candida albicans* 90028 and ATCC *Aspergillus flavus* 204304 was used for quality control of the test. The broth microdilution test was performed by using sterile, disposable, multiwell microdilution plates (96U- shaped wells). 100l of varying drug concentrations was dispensed in each rows from 1 to 10. 100l of inoculum was also dispensed. Incubated at 35^0C for 48 hours.

INTERPRETATION:
Minimum inhibitory concentration was as defined as the lowest drug concentration that showed 100% inhibition of the growth compared to growth control well.

The test was read in comparison with growth control and antibiotic control. The adequate inhibition (or) clearance of growth after 24-48 hours was taken as MIC.

RESULTS
Out of 150 patients, catheter tip and peripheral venous blood was analyzed for fungal growth. Fungi isolated from of Catheter tip 7(4.6%), 3 (2%) of peripheral venous blood samples and 8(24%) of urine samples. Parasitic infections was noted in 13(65%) patient with gastrointestinal symptoms.(Table 1)

Out of the 18(12%) isolates of fungi *C.albicans, C.tropicalis, C.parapsilosis* constituted 5.6%. All the *Candida* isolates were isolated from blood and 100% sensitive to Fluconazole and Amphotericin B. *Aspergillus flavus* 2 (11.1%) and *Aspergillus fumigatus* 1(5.6%) isolated from catheter tip showed 100% sensitive to Amphotericin B, Itraconazole, Voriconazole (Table 2 & 3)

Antifungal susceptibility testing was done by microdilution method as per the CLSI guidelines. *Aspergillus flavus* and *Aspergillus fumigatus* were sensitive to Amphotericin B, Voriconazole, Itraconazole within the MIC range. All the *Candida* isolates were sensitive to Fluconazole, Amphotericin B within the susceptibility range. None of the organisms isolated in our study had multi drug resistance. (Table 4)

Among the 150 patients 13 of them had parasitic infections, *Entamoeba histolytica* was predominantly seen in 6(46.1%) of patients with gasterointestinal symptoms followed by *Giardia* 4 (30.7%), *Cryptosporidium parvum* 2*(*15.3%) and Microsporidia 1(7.6%) (Table 5)

Diabetes mellitus 33 (94.2%), Anaemia 32 (91.4%) History of previous dialysis 31(88.5%) and Infection during the previous dialysis 28 (80%) was identified as the commonest predisdisposing factors for the occurrence of fungal and parasitic infections in patients on hemodialysis.(Table 6)

Table 1: Distribution of samples collected from study group and their culture positivity

Samples	Fungi	Parasites
Catheter tip (n= 150)	7 (4.6%)	-
Peripheral venous blood (n= 150)	3 (2%)	
Central venous catheter blood (n=9)	-	-
Swab from Infected Site (n=12)	-	-
Urine (n=33)	8 (24.2%)	
Sputum (n=15)	-	-
Stool (n=20)	-	13 (65%)

Table 2: Type of Fungal Isolates from various samples (n=18)

Fungi	Catheter tip	Peripheral venous blood	Urine
C.albicans	2 (11.1)	1 (5.6)	4 (22.2)
C.parapsilosis	1 (5.6)	1 (5.6)	1 (5.6)
C.tropicalis	1 (5.6)	1 (5.6)	3 (16.6)
Asp. Flavus	2 (11.1)	-	-
Asp.fumigatus	1 (5.6)	-	-

A total of 5.6% of *C.albicans, C.tropicalis, C.parapsilosis* was isolated from blood. *Aspergillus flavus* was isolated only from catheter in 11.1% of cases and *Aspergillus fumigatus* in 5.6% of cases. Among the 4 cases *Candida* species isolated from catheter 3 cases had candidemia.

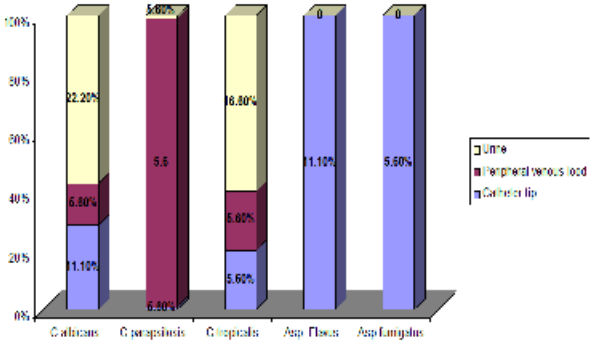

Fig 1: Type of Fungal isolates from various samples

Table 3: Anti fungal susceptibility pattern of Candida isolates

Isolate	No. of Isolates	Fluconazole		Amphotericin B	
		Sensitive	Resistance	Sensitive	Resistance
		MIC < 8	MIC>64	MIC <1	MIC>1
Candida albicans	7	7 (100)	-	7 (100)	-
Candida parapsilosis	3	3 (100)	-	3 (100)	-
Candida tropicalis	5	5 (100)	-	3 (100)	-

All the *Candida* isolates were 100% sensitive to Fluconazole and Amphotericin B.

Fig. 3 : SDA Yeast like fungi

Fig.4 : Chrom Agar Candida speciation

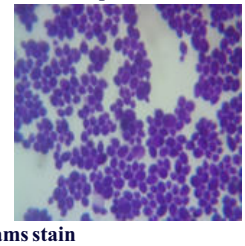

Fig:5 Candida Grams stain

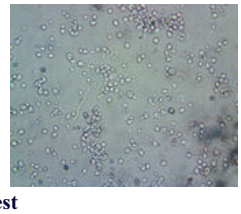

Fig.6: Germ tube test

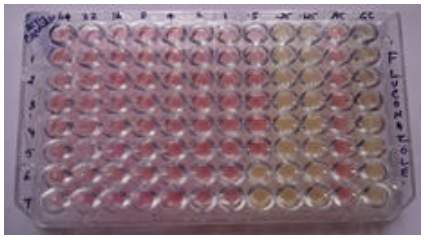

Fig.7: MIC-Fluconazole

Table 4: Anti fungal susceptibility pattern of Aspergillus species

Isolate	Amphotericin B		Itraconazole		Voriconazole	
	Sensitive MIC<2	Resistance MIC>2	Sensitive MIC<8	Resistance MIC>8	Sensitive MIC<8	Resistance MIC>8
Aspergillus flavus (n=2)	2 (100)	-	2 (100)	-	2 (100)	-
Aspergillus fumigatus (n=1)	1 (100)	-	1 (100)	-	1 (100)	-

Asp.flavus and *Asp.fumigatus* showed 100% sensitive to Amphotericin B, Itraconazole, Voriconazole

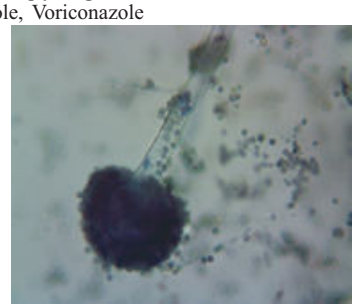

Fig.8: LPCB-Aspergillus flavus

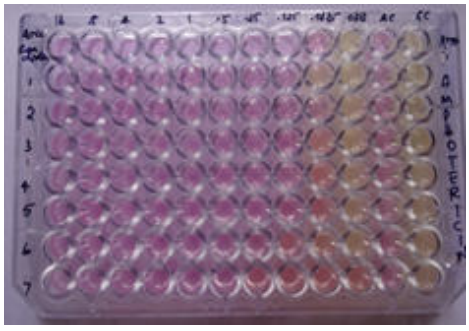

Fig.9: MIC – Amphotericin B

Table 5: Type of Parasites from Stool sample (n=13)

Parasite	No	Percentage
Entamoeba histolytica	6	46.1%
Giardia lamblia	4	30.7%
Cryptosporidium parvum	2	15.3%
Microsporidia	1	7.6%

Entamoeba histolytica was predominantly seen in 46.1% of patients with gasterointestinal symptoms followed by *Giardia* 30.7% and *Cryptosporidium parvum* 15.3%.

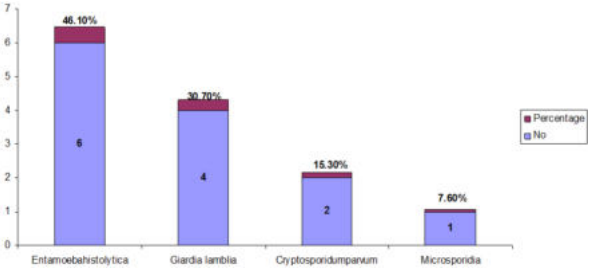

Fig.2 Type of Parasites from stool sample(n=13)

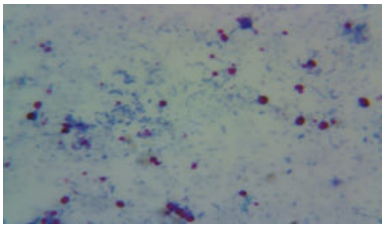

Fig 10: Modified acid Fast staining - Cryptosporidium parvum

Table 6: Type of predisposing factors for Fungal infection

Diagnosis	No of cases	Percentage
History of previous dialysis	31	88.5
History of infection in previous dialysis	28	80.0
Anaemia	32	91.4
Diabetes Mellitus	33	94.2
Hypertension	11	31.4
Hepatitis C positive	1	2.8

The predominant risk factor observed was diabetes mellitus 94.2%,followed by anaemia 91.4% .

DISCUSSION

A total of 12% fungal infections were noted in our study. Among the yeast like fungi isolated from CRBSI (5.6%) *Candida albicans, Candida parapsilosis* and *Candida tropicalis* were isolated from both catheter and blood. (11.1%) of *Aspergillus flavus*, (5.6%) of *Candida parapsilosis* and *Candida tropicalis*, and (5.6%) of *Aspergillus fumigatus* were isolated only from catheter . Ratnaja *et al* reported 41% higher risk of CRBSI and Abbot *et al* reported that 79% of *Candida* infection occurs in early hemodialysis especially in patients with diabetes mellitus and anaemia.[5] Our study is similar to the study of Hoen *et al* and Gandhi *et al*[1] in isolating fungal infections. Ramanathan *et al* reported (16%)of *Candida albicans* were isolated from central venous catheters in patients on HD.[7] Bernad *et al* [8], Monina *et al* [9] reported in 1.7% of *Candida albicans* infections in central venous catheters. In our study (16.6%) *Candida tropicalis*, (22.2%) *Candida albicans* and (5.6%) of *Candida parapsilosis* were isolated from urine samples.

Antifungal susceptibility testing was done by microdilution method as per the CLSI guidelines. *Aspergillus flavus* and *Aspergillus fumigatus* were sensitive to Amphotericin B, Voriconazole, Itraconazole within the MIC range. All the *Candida* isolates were sensitive to Fluconazole, Amphotericin B within the susceptibility range. No fungus was isolated from Swab and Sputum. None of the organisms isolated in our study had multi drug resistance.

Parasitic infection was noted in 8.6%. The predominant parasite was *Entamoeba histolytica* (41.6%), *Giardia lamblia* (30.7%), *Cryptosporidium parvum* (15.3%) of cases and *Microsporidia* (7.6%) cases. No malarial parasites were detected in the study group. Parasitic infections were noted in patients who were in an immunosuppressed state, diabetics and elderly age group. Seyrafian *et al* [8, 9,10, 11] stated that 43.9%of HD patients infected with intestinal parasitic infection. Our study correlates with their study in detecting the parasitic infection in patients on HD. Kulik *et al* [2]and Seyrafian *et al*[11] stated the prevalence of *Blastocystis spp* (18%) was commonly noted in patients with renal impairment. *Endolimax nana* (14%), *Cryptosporidium parvum* (4 - 4.7%) and *Entamoeba coli* (4%) and (1.2%)*Giardia* were noted in patients with high uremic state [13, 18]. He stated that high uremic state and altered immune mechanism, neutropenia in HD patients promotes the parasitic infection and worsen the state of aneamia and aggravates the state of immunosuppression. Our study correlates with the study of Seyrafian *et al* [11] in demonstrating the parasites in patients with renal impairment.

The predominant cause among patient with CKD was Diabetes mellitus (35%), followed by Hypertension (32%). 14.2% patients had post renal transplant failure. Among the 44 of AKI 50% had Medical renal disease less than 3 months and 16.1% had acute diarrheal disease [1, 13, 26].

CONCLUSION

Considering the immunosuppressive state of patients on hemodialysis, screening for fungal and parasitic infection in such patients should be mandatory. Site, duration and number of catheterization play a major role in development of fungal and parasitic infections. Apart from diabetes mellitus and anaemia, history of infection in the previous dialysis and the number of dialysis were the common predisposing factors for fungal and parasitic infections. Risk of Candidemia can be greatly reduced by strict aseptic technique. This study gave an insight on strict hand washing, changing of catheters with sterile precaution. Health education regarding taking care of catheter and improving the general condition is essential in preventing fungal and parasitic diseases.

REFERENCES:

[1] BV Gandhi, MM Bahadur, H Dodeja, V Aggrwal, A Tamba, M Mali, Systemic fungal infections, *Journal of Post Graduation Medicine 2005,* Vol: 51, Issue:5, Page 32-36
[2] Rose Anne Kulik, Dina Lucia, Morasis Falavigna, Leticia Nishi and Silvana Marques Araujo, Blastocystis sp. and other Intestinal Parasites in Hemodialysis Patient, *The Brazillian Journal* of Infectious Disease, 2008;12(4) 338
[3] Kinchen KS, Sadler J, Fink N. The timing of specialist evolution in chronic kidney disease and mortality. *Ann Intern Med* 137:479-486, 2002.
[4] B Lynn Johnston, MD and John MConly MD, Central Venous catheter- associated bloodstream infection n hemodialysis patients: Another patient safety bundle? *Can J infect Dis Med Microbiol*. 2006 Mar-Apr, 17(2):
[5] Abott KC, Hypolite I,. Hospitalizations for fungal infections after initiation O: States. Nephron 2001;89:426-32
[6] Tokars JI *et al*.(2001) A prospective study of vascular access infections at sevan outpatient hemodialysis enters. *Am J Kidney* Dis 37: 1232-40
[7] Ramanathan Paramewaran, jatan B. Shrchan, Muralidhar Varma D, Chiranjay, Mukhopadhyay, Sudha Vidyasagar Intravascular catheter – related infections in an Indian tertiary care hospital. J Infect Dev Ctries 2011; 5 (6):452-458.
[8] Bernard Canaud. Heaemodialysis catheter related infection: time for action, *Nephrol Dial Transplant* (1999) 14:2288-2290
[9] Monina Klevens.R, Jonathan R.Edwards, Mary L.Andrus, Kelly D.Peterson, Margaret A. Dudeck, Teresa C.Horan and the NHSN Participants in Outpatient Dialysis Surveillance.
[10] Dialysis Survelillance Report: National Health Care safety Network (NHSN)2006. Division of Health care quality promotion, centers for Disease control and prevention, seminars in Dialysis –Vol 21, No.1 2008; p.24-28
[11] Seyrafian SH, Pestechian N, Kerdegari M, Yousefi HA, Bastani B, Prevalence rate of cytptosporidium infection in hemodialysis patients in Iran. hemodialysis International 2006;10:375-9.
[12] Evering T, Weiss L. The immunology of parasite infections in immunocompromised hosts. *Parasite Immunol*. 2006; **28**: 549–565.
[13] Stark D, Barratt J, Van Hal S, Marriott D, Harkness J, Ellis J. Clinical significance of enteric protozoa in the immunosuppressed human population. *Clin Microbiol Rev*. 2009; **22**: 634–650.
[14] Hassan NA, Lim YAL, Mahmud R, et al. Microsporidia infection among various groups of the immunocompromised patients. *Trop Biomed*. 2018; **35**: 521–530.
[15] Hunter PR, Nichols G. Epidemiology and clinical features of *Cryptosporidium* infection in immunocompromised patients. *Clin Microbiol Rev*. 2002; **15**: 145–154.
[16] Omrani VF, Fallahi S, Rostami A, et al. Prevalence of intestinal parasite infections and associated clinical symptoms among patients with end□stage renal disease undergoing hemodialysis. *Infection*. 2015; **43**: 537–544.
[17] Hawash YA, Dorgham LS, Amir E□AM, Sharaf OF. Prevalence of intestinal protozoa among Saudi patients with chronic renal failure: A case□control study. *J Trop Med*. 2015; **2015**: 1–9.
[18] Kulik RA, Falavigna DLM, Nishi L, Araujo SM. Blastocystis sp. and other intestinal parasites in hemodialysis patients. *Brazilian Journal of Infectious Diseases*. 2008; **12**: 338–341.
[19] Luke RG, Boyle JA. Renal effects of Amphotericin B lipid complex. Am J Kidney Dis1998;31:780-5
[20] Turkcapar N, Kutlay S, Nergizoglu G, Atli T, Duman N. Prevalence of *Cryptosporidium* infection in hemodialysis patients. *Nephron*. 2002; **90**: 344–346.
[21] Yücel A, Bulut V, Yılmaz M. Investigation of *Cryptosporidium* spp. in patients with diarrhea or undergoing haemodialysis in the region of Elazığ. *Türk Parazitol Derg*. 2000; **24**: 126–132.
[22] Ferreira□Filho SR, da Costa Braga FC, de Sa DM, et al. *Entamoeba histolytica/Entamoeba dispar* infection in chronic hemodialysis patients. *Saudi J Kidney Dis Transpl*. 2011; **22**: 237–244
[23] Tonelli M, Wiebe N, Culleton B, et al. Chronic kidney disease and mortality risk: A systematic review. *J Am Soc Nephrol*. 2006; **17**: 2034–2047.

[24] George IIJ, V. Shankar. Epidemiology of systemic mycoses among renal transplant recipients in India. Transplantation 2003;75:1544-51
[25] Chugh KS, *et al* . High mortality in systemic fungal infection following renal transplantation in 3rd world countries, NDT. 1993;8:168
[26] Kato S, Chmielewski M, Honda H, et al. Aspects of immune dysfunction in end□stage renal disease. *Clin J Am Soc Nephrol*. 2008; **3**: 1526–1533.