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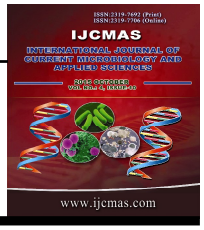

Original Research Article

Intestinal Parasitic Infections in Renal Allograft Recipients-Necessity of Periodic Screening and Prudent Choice of Diagnostic Techniques- A Prospective Study

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A B S T R A C T

Renal allograft recipients are maintained on lifelong immunosuppression. They are predisposed to infections. In the setting of commoner bacterial and viral infections, intestinal parasitic infections get overlooked. This is hazardous and prompt laboratory diagnosis of such infections is necessary. This study was initiated to determine the prevalence of intestinal parasitic infections in renal allograft recipients. Stool specimens from 206 adult renal allograft recipients were examined for intestinal parasites using Wet Mount, Flotation technique of saturated salt solution, Formol Ether Sedimentation, Modified Kinyoun's acid fast staining and Trichrome staining of Wheatley. Intestinal parasites were detected in 30.58% of renal allograft recipients. Common intestinal protozoa, coccidian parasites and helminths were detected in 28.66%, 0.96% and 0.96% of renal allograft recipients respectively. 22.59% of asymptomatic cases harboured intestinal parasites. For asymptomatic cases, least 2 stool specimens were necessary to achieve acceptable sensitivity while for symptomatic cases a single specimen suffices. Intestinal parasitic infections are quite prevalent in renal allograft recipients and may be symptomatic or asymptomatic. Hence, all renal transplant recipients should be screened routinely for the same.

Introduction

Parasites are incredibly diverse, astonishingly successful as well; hence, extremely prevalent in immuno-compromised individuals. This is more so with opportunistic parasites which are normally innocuous but, gain strong foothold in a setting of immunosuppression.

In recent years, proportion of immuno-compromised patients such as organ transplant recipients and those having HIV/AIDS, diabetes, malignancies and those on chemotherapy is on the rise (Gan, 2006; Narayan *et al.*, 2006; Sharp and Hahn, 2011; Salomon *et al.*, 2012). Among organ

transplantations, renal transplantation is more prevalent because of high proportion of Chronic kidney disease cases (CKD). CKD generally progresses to end stage renal disease (ESRD) and such patients have to undergo renal transplantation (Veerappan, 2013; Anon, 2002). Patients who have undergone renal transplantation are maintained on lifelong immunosuppression. This makes them more susceptible to infection. Infections are the second commonest cause of death in renal transplant and commonest cause of death during the first year after transplant (Fishman, 2008). In the setting of commoner bacterial and viral infections (Fishman, 2008), intestinal parasitic infections get overlooked and this might be hazardous. In immunocompromised patients, intestinal parasitic infections may be asymptomatic which poses a threat to the patient as well as to the community. Symptomatic parasitic infections can lead to serious morbidity and even mortality (Azami *et al.,* 2004; Al-Megrin, 2010; Naeini *et al.,* 2012). Thus, making laboratory diagnosis of these infections is mandatory.

Hence, the present study was initiated to determine the prevalence of intestinal parasitic infections in post renal transplant patients.

Material and Methods

Study design: Prospective study

Selection of participants: After obtaining Institutional Ethics Committee approval a total of 206 patients aged 18 years or more and who had received renal transplant 2 weeks back or earlier were recruited for the present study. Written informed consent was obtained from them. The study was carried out for one year at a tertiary care multi-specialty teaching institute. Patients who had received anti-parasitic treatment in the last two weeks were excluded from the study.

Methodology: Clinical history was obtained especially with respect to patient particulars, time elapsed since transplant, immunosuppressive regimen, gastro-intestinal signs and symptoms and other relevant data if any. The patients were instructed to submit three stool specimens in leak proof, dry, wide mouthed plastic containers on non-consecutive days. The stool specimens were examined with respect to gross examination and microscopic examination using wet mount, flotation technique of saturated salt solution and formol ether sedimentation, modified Kinyoun's acid fast staining on the concentrated deposit and trichrome staining of Wheatley (Garcia, 2007).

Patients detected with intestinal parasitic infections were referred to the clinician for appropriate treatment and asked to submit three stool specimens on non-consecutive days after treatment.

Statistical analysis: The results were observed, interpreted and recorded and the data was analyzed using the Chi square statistic wherever applicable. $P < 0.05$ was considered significant. The final result of all techniques has been considered to be the gold standard.

Results and Discussion

63 out of 206 renal transplant recipients (30.58%) harboured intestinal parasites. There was no significant age or gender predilection noted among these patients.

41.26 % (85/206), 33.98 % (70/206) and 24.76 % (51/206) transplant recipients submitted three, two and one stool specimen/s respectively. Sensitivity of two stool specimens to detect intestinal parasites (95.23%) was significantly higher than that

of a single stool specimen (47.61%)(P value = 0.00000049). A third stool specimen did not provide any significant increase in yield (P = 0.12) (Figure 1).

Of 206 patients 29 were symptomatic whereas 177 were asymptomatic. Intestinal parasites were detected in 79.31% (23/29) of symptomatic patients and 22.59% (40/177) of asymptomatic. Detection of parasites in the 1st stool specimen was significantly higher in symptomatic patients as compared to asymptomatic patients (P = 0.00002) while detection of parasites only in the 2nd stool specimen was significantly higher in asymptomatic patients as compared to symptomatic patients (P = 0.00003) (Table 1).

Difference in prevalence of intestinal parasitic infections within three months (25.64%) and after three months (31.73%) of renal transplant was insignificant (P value = 0.45) (Table 2).

59 out of 206 (28.66%) transplant recipients were found to be infected with common intestinal protozoa (*Entamoeba histolytica/dispar, Entamoeba coli, Giardia lamblia and Blastocystis hominis*) (Table 3). Two out of 206 (0.96%) transplant recipients were found to be infected with helminths which comprised *Ascaris lumbricoides* and hookworm. Two out of 206 (0.96%) transplant recipients were found to be infected with coccidian parasites which comprised *Cryptosporidium* species and *Cystoisospora belli*. Both were symptomatic. Sensitivities of techniques to detect intestinal protozoa, direct wet mount, wet mount after formol ether sedimentation technique and trichrome staining were 61.95%, 95.65% and 100% respectively (Table 4). No intestinal parasites were detected in repeat stool specimens of patients who were treated for the same.

The overall prevalence of intestinal parasitic infections in our study was 30.58%. No age or gender predilection was noted. Standard guidelines recommend that 3 stool specimens must be collected on non-consecutive days for detection of parasites in stool (Garcia, 2007). However in the present study a second stool specimen provided a significant increase in yield but, a third specimen failed to provide any added advantage (Figure 1).

22.59% (40/177) of asymptomatic patients harboured intestinal parasites (Table 1). Renal allograft recipients are frequently asymptomatic since immunosuppression delays inflammatory responses (Stark *et al.*, 2009). Intermittent shedding of parasites in asymptomatic patients leads to lesser detection too (Kang *et al.*, 1998). This might be risky to the patient and community (Pellegrino, 2012; Cruz, 2104). Hence, it is necessary to screen all renal allograft recipients for intestinal parasitic infections. In symptomatic patients a single stool specimen may provide reasonable sensitivity while in asymptomatic patients, examination of 2 specimens is a must.

All renal transplant recipients receive lifelong immunosuppressive regimen which is tapered off to a minimum level by 3 months. Thus, the recipient is maximally immunosuppressed during the first 3 months after transplant. But in our study, there was no correlation between prevalence of intestinal parasitic infections and the time elapsed since transplant (P = 0.45) (Table 2). Comparison with Indian data was not possible due to lack of the same. Protozoa dominated the list of parasites detected in this study (28.66%) (Table 3).

Entamoeba histolytica/dispar was the predominant protozoan (11.67%) followed by *Giardia lamblia* (8.26%). Other studies too have suggested that protozoal infections are commoner inrenal allograft

recipients.(Azami *et al.,* 2004; Naeini *et al.,* 2012; Rostami *et al.,* 2007)Prevalence of *Entamoeba coli* infection was found to be 0.96% (2/206) in our study. Although *Entamoeba coli* is considered to be a non-pathogen, it indicates a state of general lack of hygiene (Faulkner *et al.,* 2003). Its detection in stool indicates that personal hygiene should be stringently stressed upon.

Table.1 Distribution of symptomatic and asymptomatic renal allograft recipients

	Symptomatic	**Asymptomatic**	**Total**
Parasites detected in 1st specimen	19	11	30
Parasites detected in 2nd specimen	3	27	30
Parasites detected in 3rd specimen	1	2	3
Parasites not detected	6	137	143
Total	29	177	206

Table.2 Time elapsed since transplant and intestinal parasitic infections

	Parasites detected	**Parasites not detected**	**Total**
2weeks - 3months	10(25.64%)	29(74.36%)	39
After 3 months	53(31.73%)	114(68.27%)	167
Total	63(30.58%)	143(69.42%)	206

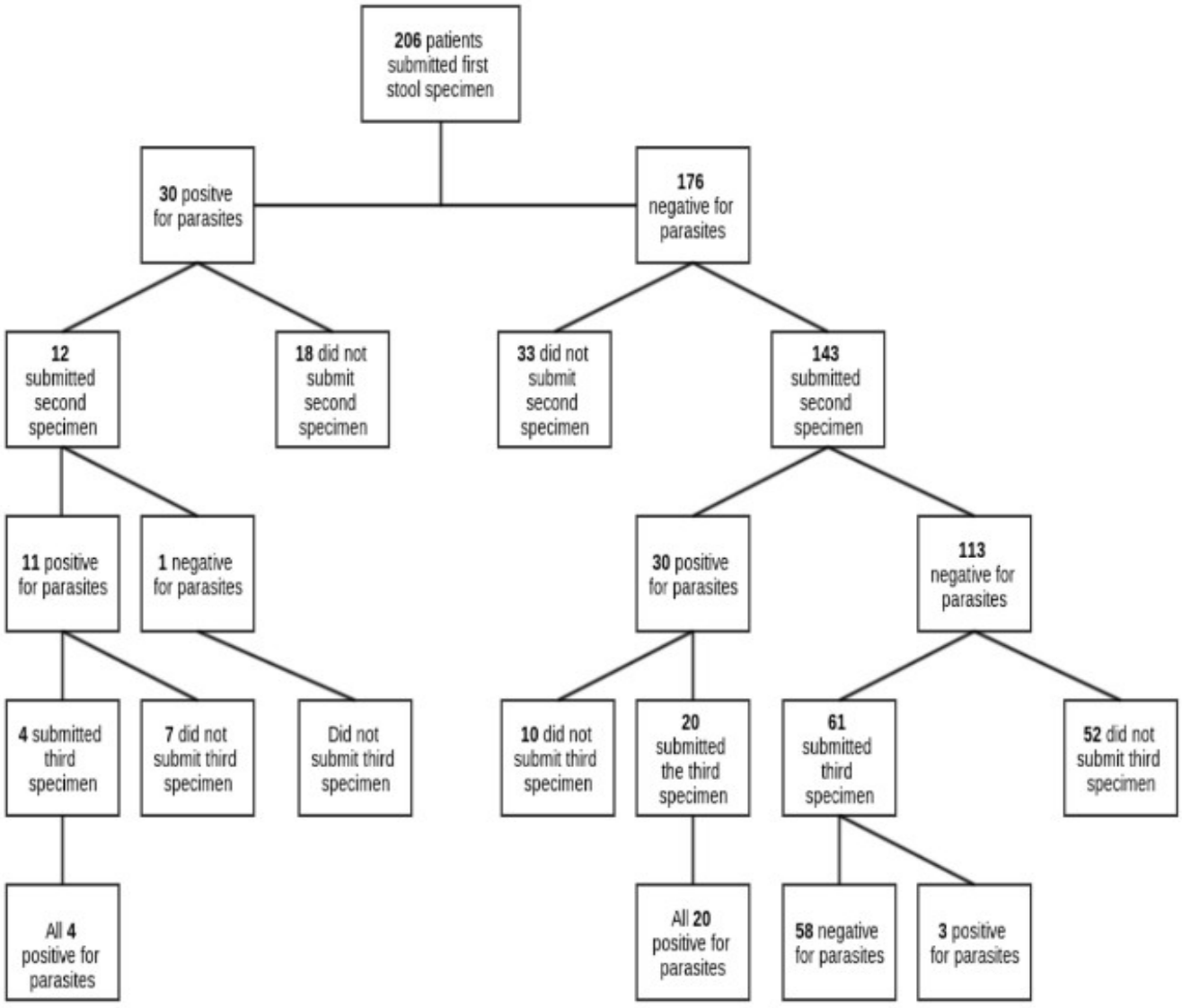
Table.3 Individual parasites and their prevalence

	Parasite	**Number (%)**
Common Intestinal Protozoa	*Entamoeba histolytica/dispar*	24(11.67%)
	Entamoeba coli	2(0.96%)
	Giardia lamblia	17(8.26%)
	Blastocystis hominis	7(3.40%)
	Mixed protozoal infections	9(4.37%)
Helminths	Ova of hookworm	1(0.48%)
	Fertilized ova of *Ascaris lumbricoides*	1(0.48%)
Coccidian parasites	*Cryptosporidium* species	1(0.48%)
	Cystoisospora belli	1(0.48%)

Table.4 Comparison of techniques for the detection of common intestinal protozoa

Technique of detection	Protozoa detected	Sensitivity
Direct wet mount	57	61.95%
Formol ether sedimentation	88	95.65%
Trichrome staining	92	100%

Figure.1 Specimens submitted and parasites detected



Mixed parasitic infection is a common problem in the developing countries. In our study, mixed protozoal infection was seen in 4.37% (9/206) of patients. Combined infections can exacerbate clinical manifestations. Hence, prompt detection is warranted.

In our study, the prevalence of helminthic infections was0.96% (2/206) (Table 3)

which is similar to findings of Azami *et al.* (2010) and Rostami *et al.* (2007). Coccidian parasites were detected in 0.96% (2/206). In recent times, prevalence of cystoisosporiasis has reduced due to prophylactic trimethoprim-sulfamethoxazole for the prevention of pneumonia due to *Pneumocystis jirovecii* (Tolan, 2013). Our study group was also being maintained on lifelong trimethoprim-sulfamethoxazole prophylaxis, hence the low prevalence of cystoisosporiasis.

In the detection of common intestinal protozoa, which were predominant parasites in our study, sensitivity of formol ether sedimentation technique (95.65%) was clearly superior to that of direct wet mount (61.95%). Trichrome staining of Wheatley exhibited a sensitivity of 100% for common intestinal protozoa (Table 4). Although formol ether sedimentation technique is not as sensitive as trichrome staining, it is a rapid and cheap alternative to trichrome staining in a resource limited setup.

Transplant recipients who harboured intestinal parasites were treated for the same. Repeat stool examination revealed no parasites.

Significance of results: From our study it is evident that post renal transplant patients constitute a distinct group of immunosuppressed patients. It is the common intestinal protozoa that primarily cause infection in these patients. Choice of examination technique should be prudent and decided as per clinical presentation. Prompt diagnosis and timely management is indeed capable of reducing morbidity and mortality associated with intestinal parasitic infections in renal allograft recipients.

Out of the9 mixed protozoal infections, 5 were due to *Entamoeba histolytica/dispar* and *Blastocystis hominis*, 2 were due to *Blastocystis hominis* and *Giardia lamblia*, 1 was due to *Entamoeba histolytica/dispar* and *Giardia lamblia* and was due to *Entamoeba histolytica/dispar* and *Entamoeba coli*

Total no. of specimens in which protozoa were detected = 92 (taking into account 446 specimens submitted by 206 patients). In 30 patients parasites were detected in 1st specimen. A 2nd specimen allowed the detection of parasites in 30 more patients. In 3 patients, parasites were detected in the 3rd specimen only.

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