Original Article Indian J Pediatr 1999; 66 : 21-26

Role of Protozoa as Risk Factors for Persistent Diarrhea

Nita Bhandari, Rajiv Bahl, Tarun Dua, Ramesh Kumar and Ranjana Srivastava

ICMR Advanced Centre for Diarrheal Disease Research, Department of Pediatrics, All India Institute of Medical Sciences, New Delhi

Abstract : A case control study including 175 children aged 0-36 months suffering from diarrhea of ≥ 14 days duration was undertaken to determine whether there is an association between *Giardia lamblia*, *Entamoeba histolytica* or *Cryptosporidium* infection and persistent diarrhea (PD). Subjects were identified by ongoing household surveillance and enrolled as cases. For each case two controls were selected by survey of neighbouring households - a child with acute diarrhea and one without diarrhea. Both the controls were matched with the case for age and nutritional status. Two fresh stool samples were collected from all cases and controls at enrolment and examined for trophozoites of *Giardia lamblia*, *Entamoeba histolytica* and *Cryptosporidium*.

Giardia lamblia trophozoites were detected in a significantly higher proportion of PD cases (20.0%) than acute diarrheal and non diarrheal controls (4.6% each, $p < 0.0001$). There were no significant differences in the proportion of cases and controls who passed *E. histolytica* trophozoites or cryptosporidium in their stools. There was a consistent trend towards poorer weight gain in PD cases who passed Giardia trophozoites in stool; the differences were statistically significant at days 14 and 21, after enrolment.

Giardia lamblia infection is more prevalent in PD cases than in acute diarrhea or non-diarheal controls. This prevalence is not high enough to warrant routine anti-giardia therapy in patients with PD. However, as giardiasis was observed to have adverse growth impact in PD cases, stool microscopy for detection and subsequent treatment of *Giardia lamblia* seems to be justified. **(Indian J Pediatr 1999; 66 : 21-26)**

Key words : *Persistent diarrhea; Giardia lamblia; Entamoeba histolytica.*

Persistent diarrhea (PD) has emerged as an important public health problem in developing countries as it contributes to a substantial portion of the nutritional decline and mortality associated with diarrheal illness[1-4].

Until recently, the role of pathogens in the etiology of this disorder was unclear. Viruses are not commonly isolated from stools of children with persistent episodes of diarrhea. Enterotoxigenic and enteropathogenic *Escherichia coli*, aeromonas, campylobacter and shigella species are detected in both acute and persistent episodes but are not particularly associated with PD[5]. Enteroadherent *E. coli* have been isolated more frequently in the early phase of persistent episodes than in acute diarrheal episodes in India[6], Bangladesh[7] and Mexico[8]. In other studies, the frequency of isolation was similar in acute and persistent episodes[9-11]. Since the pathogens implicated in PD were detected in stools of less than half the patients in most studies,

Reprint requests : Nita Bhandari, Department of Pediatrics, All India Institute of Medical Sciences, New Delhi-110 029.

search for other etiological agents must continue. In this regard, the role of parasites and protozoal agents needs further investigation.

Among protozoa, the detection rates of *Giardia lamblia* in persistent and acute diarrhea have varied widely. Some studies have indicated an association of PD with giardiasis[9,10] while others have failed to show higher excretion rates of *G. lamblia* in PD than in acute diarrhea[6,7]. However, several of these latter studies included patients who had already received antigiardia therapy before stool examination. The role of *Entamoeba histolytica* and cryptosporidium in PD among children in developing countries is not yet defined. This case control study was conducted to determine whether there is an association between protozoal agents and PD.

METHODS AND MATERIALS

The study was undertaken in the urban slum of Tigri in New Delhi which has a population of about 30,000. There was an ongoing household diarrheal surveillance system for another study being conducted by the same team. This surveillance system identified all children aged < 3 years suffering from diarrhea.

Diarrhea was defined as the passage of three or more liquid stools in a 24 hour period. Persistent diarrhea was defined as diarrhea lasting ≥ 14 days. Acute diarrhea was defined as diarrhea of < 14 days duration.

Children aged 0-36 months suffering from diarrhea of ≥ 14 days with not more than two diarrhea free days in between were cases identified through surveillance. For each case, two sets of controls were selected by survey of neighbouring households : a child with acute diarrhea and one without diarrhea. Both the controls were matched with the case for age (< 6, 7-12, 13-24 and 25-36 months) and nutritional status category [weight for age ≤ 80% or > 80% of the United States National Centre for Health Statistics (NCHS) Median].

The acute diarrheal control was the first child identified with diarrhea of < 14 days in the same age and nutritional status category as the case, and residing among the neighbourhood household.

The non diarrheal control was one who had not suffered from diarrhea in the previous 6 weeks (according to the caretaker) and did not develop diarrheal symptoms during the week after selection. This control was recruited by visiting household numbers in ascending order beginning from the dwelling adjacent to that of the case, and selecting the first appropriate age and nutritional category matched child. This selection of controls was usually accomplished on the same day.

A follow up visit was made by field workers on the day coinciding with day 14 of the acute diarrheal episode and day 7 after enrolment of the non diarrheal control. If an acute episode became persistent or a non-diarrheal child developed diarrhea, these children were excluded and new controls selected.

The parents of all selected children were informed of the purpose of the study and consent was obtained. Socio-economic details of the household were obtained.

All children were weighed at enrolment; additionally, children having PD were weighed on days 7, 14 and 21, and four weeks after recovery from diarrhea.

Two fresh stool samples were collected at enrolment and examined by a trained laboratory technician within 30 minutes in

the field laboratory. Stool microscopy was done for *Entamoeba histolytica* and Gardia trophozoite detection. A small amount of stool was mixed with a drop of 0.85% saline in Lugol's iodine and seen under high power of the light microscope. One third of the specimens were transferred to the central laboratory at the All India Institute of Medical Sciences (AIIMS) immediately, where they were examined independently by a microbiologist. A 94% agreement was obtained between the two observers.

For cryptosporidium detection, a standard smear that was not too thin was prepared. It was passed over a flame once and fixed in 3% hydrochloric acid in methanol for 5 minutes. Modified Ziehl Neelson technique was adapted for staining of the slides using Kinyoun's stain. The stained slides were transported to the central laboratory at AIIMS where they were examined independently by a microbiologist.

Analysis was done using EPI INFO and SPSS PC+ software. Cases and controls were compared for categorical variables using the chi-square test. Quantitative data was compared using ANOVA & Kruskal Wallis test was used when the variances were not homogenous.

RESULTS

The baseline characteristics of the case and controls were similar (Table 1). *Giardia lamblia* trophozoites were detected in a significantly higher proportion of PD cases (20.0%) than acute diarrheal and non diarrheal controls (4.6% each; $p < 0.0001$). When children were stratified by nutritional status, giardia trophozoites in stool were detected in a significantly higher proportion of PD cases than AD or non diarrheal controls in both well nourished and malnourished children (Table 2). There were no significant differences in the proportion of cases and controls who passed *E. histolytica* trophozoites or cryptosporidium in their stools (Table 2).

To study the growth implications of the association of PD with giardia infestation,

TABLE 1. Baseline Comparison of Cases and Controls

Characteristics	Persistent diarrhea cases (PD) (n = 175)	Controls	
		Acute diarrhea (n = 175)	Without diarrhea (n = 175)
Mean age (SD)	14.5 (10.1)	14.5 (8.8,	17.7 (8.9)
Males (%)	45.7	54.9	37.7
Mean weight for age (SD) as % of NCHS median	75.4 (12.7)	74.6 (12.2)	74.3 (10.7)
Breast fed (%)	66.3	72.0	66.9
Literate (%)			
Mothers	20.0	13.7	12.0
Fathers	47.4	38.3	40.6
Water supply from hand pumps (%)	98	99	98
Use of community toilets (%)	95	97	95

TABLE 2. Prevalence of Protozoa in Cases and Controls

	n	Giardia trophozoites number (%)	Entamoeba histolytica trphozoites number (%)	Crypto-sporidium number (%)
Malnourished (WFA ≤ 80% of NCHS median)				
Persistent diarrhea cases	115	23 (20.0)	9 (7.8)	2 (1.7)
Acute diarrhea controls	115	4 (3.5)**	6 (5.2)	2 (1.7)
Non diarrheal controls	115	5 (4.3)**	6 (5.2)	6 (5.2)
Well nourished (WFA > 80% of NCHS median)				
Persistent diarrhea cases	60	12 (20.0)	2 (3.3)	1 (1.7)
Acute diarrhea controls	60	4 (6.7)*	1 (1.7)	5 (8.3)
Non diarrheal controls	60	3 (5.0)*	2 (3.3)	3 (5.0)

* $p < 0.05$; ** $p < 0.0001$

TABLE 3. Post Enrolment Mean Weight Gain (as % of Baseline Weight) Among Persistent Diarrhea Cases

Between enrolment and	% weight gain (Mean SD)		p value
	Giardia trophozoites present n = 33	Giardia trophozoites and cysts absent# n = 122	
Day 7	0.6 (3.5)	1.6 (3.2)	0.13
Day 14	10 (3.3)	3.3 (4.4)	0.007
Day 21	1.3 (3.6)	4.5 (5.9)	0.01*
1 month post recovery	3.7 (5.0)	6.1 (7.9)	0.15*

cases with only giardia cysts (n = 20) excluded from this analysis
* non parametric test used

we compared the mean weight gain in PD cases with and without giardia trophozoites on days 7, 14 and 21 after enrolment, and one month post recovery. There was a consistent trend towards poorer weight gain in PD cases who passed giardia trophozoites in stool; the differences were statistically significant on post enrolment days 14 and 21 (Table 3).

DISCUSSION

The results of the present study indicate that giardiasis is a significant etiological factor in persistent diarrhea. A greater proportion of children with persistent diarrhea

(20%) had giardia infection as compared to acute diarrhea (4.6%) and non diarrheal (4.6%) controls; this was true both for malnourished and well nourished children. Among children with persistent diarrhea, those who had giardia trophozoites in stools had a lower weight gain during the three week period following enrolment as compared to those negative for this pathogen.

The prevalence of giardiasis in PD in previous studies varied from 4.1% in Bangladesh[7,8] to 42.9% in Peru[9]. Giardia identification rates were similar in acute and persistent episodes in these studies. Another study from Gambia reported 45% prevalence in malnourished children with chronic diarrhea; this was significantly higher than prevalence in healthy controls but similar to marasmic controls[12].

We did not find *Entamoeba histolytica* to be associated with PD. These findings are in agreement with most previous reports[6-9].

The present study did not observe the association of cryptosporidium with PD. Cryptosporidium was detected significantly more often in children with persistent diarrhea in a previous study in Bangladesh[8] but in Peru, it was more frequently associated with acute rather than persistent episodes[9]. The prevalence of *Cryptosporidium* in diarrhea was low in the present study as in other studies that have reported 1-7% prevalence[8,9]. However, in some parts of Africa, *cryptosporidium* is associated with a substantially higher proportion of diarrhea cases[13]. It is now recognized as an important intestinal parasite associated with severe, life threatening diarrhea in immunocompromised hosts, as well as common source outbreaks of diarrhea in children without immune deficiency[14].

A natural history study of Giardia infection in infants and children in rural Guatemala reported a lower weight velocity in the second year of life in giardia infected than in giardia negative children[15]. Our study supports the findings of the Guatemalan study.

Some important limitations of our study need to be emphasized. The poor growth observed in patients with giardiasis may not necessarily be causal and could just be a continuation of a pre-illness trend. The association between PD and giardiasis may have resulted from both having a common causative factor. Stool microbiology for bacterial and viral pathogens, which may have confounded this association was not performed.

The prevalence of giardia in PD in the present study was about 20%, which is not high enough to warrant routine antigiardia therapy in patients with this disorder. However, as giardiasis was observed to have an adverse growth impact in children with PD, stool microscopy should be routinely performed in these patients and if giardia trophozoites are detected, effective treatment should be provided.

Acknowledgements

We acknowledge the financial support for this study from the World Health Organization, Geneva, and core support to the unit from the India Council of Medical Research.

REFERENCES

1. Bhan MK, Arora NK, Ghai KR, Khoshoo V, Bhandari N. Major factors in diarrhoea related mortality among rural children. *Indian J Med Res* 1986; 83 : 9-12.
2. Bhandari N, Bhan MK, Sazawal S. Mortality associated with acute watery diarrhea, dysentery, and persistent diarrhea

in rural north India. *Acta Paediatr* 1992; 81 (supple 381) : 3-6.
3. Fauveau V, Henry FJ, Briend A, Yunus M, Chakraborty J. Persistent diarrhea as a cause of childhood mortality in rural Bangladesh. *Acta Paediatr* 1992; 81 (suppl 38) : 12-14.
4. Victora CG, Huttly SR, Fuchs SC *et al.* International differences in clinical patterns of diarrheal deaths : a comparison of children from Brazil, Senegal, Bangladesh, and India. *J Diarrheal Dis Res* 1993; 11 : 22-29.
5. World Health Organization. Persistent diarrhea in children in developing countries - report of a WHO meeting. WHO/CDD/88.27.
6. Bhan MK, Bhandari N, Sazawal S *et al.* Descriptive epidemiology of persistent diarrhea among young children in rural northern India. *Bull WHO* 1989; 67 : 281-288.
7. Henry FJ, Udoy AS, Wanky CA, Aziz K. Epidemiology of persistent diarrhea and etiologic agents in Mirzapur, Bangladesh. *Acta Paediatr* 1992; 81 (suppl 381) : 27-31.
8. Baqui AH, Sack RB, Black RE *et al.* Enteropathogens associated with acute and persistent diarrhea in Bangladesh children under five years of age. *J Infect Dis* 1992; 166 : 792-796.
9. Lanata CF, Black RE, Gill A *et al.* Etiologic agents in acute persistent diarrhea in children under three years of age in peri urban Lima, Peru. *Acta Paediatr* 1992; 81 (suppl 381) : 32-38.
10. Cravioto A, Tello A, Navarro A *et al.* Association of *Escherichia coli* HEp-2 adherence patterns with type and duration of diarrhea. *Lancet* 1991; 337 : 262-264.
11. Arthur JD, Bodhidatta L, Echeverria P, Phuphaisan S, Sakti P. Diarrheal disease in Cambodian children at a camp in Thailand. *Am J Epidemiol* 1992; 135 : 541-551.
12. Sullivan PB, Marsh MN, Phillips MB *et al.* Prevalence and treatment of giardiasis in chronic diarrhea and malnutrition. *Arch Dis Child* 1990; 65 : 304-306.
13. Mølbak K, Wested N, Højlyng N *et al.* The etiology of early childhood diarrhea. A community trial from Guinea Bissau. *J Infect Dis* 1994; 169581-7.
14. Gracey M. Diarrheal disease in perspective. In : Diarrheal Disease - Nestle Nutrition Workshop Series, Volume 38, 1997, Lippincott-Raven, Philadelphia, New York.
15. Farthing MJG, Mata L, Urutia JJ, Kronmal RA. Natural history of Giardia infection of infants and children in rural Gautemala and its impact on physical growth. *Am J Clin Nutr* 1986; 43 : 395-405.

BREAST MILK : REVISITED

Human milk has been shown to be the ideal source of nutrition for most growing infants. Its composition continues to be an active area of investigation. In several studies in preterm and term infants, long chain polyunsaturated fatty acids are found to improve the maturation of visual evoked potential. The clinical significance of this finding, however, remains unclear. Nucleotides present in breast milk or added to infant formula seem to enhance the humoral immune response to vaccination. Whether breast - feeding protects susceptible infants from the risk of development of diabetes mellitus type I is still controversial. Breast feeding by mothers infected with HIV is not recommended. Other viruses and pollutants have also been found in breast milk. The importance of these in the long-term health of children remains to be established.

Abstracted from : Mayo Clin Proc 1998; 73 : 760-763