

GIARDIA-INFECTION AMONG SUBJECTS REVEALED DIARRHEA OR PASSAGE OF SOFT STOOL IN KOCHI PREFECTURE, JAPAN

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Abstract: Prevalence of *Giardia* spp. infection was determined among subjects attending Kochi Medical School Hospital from April 1996 to January 1998. Cysts of *Giardia* spp. were found in 10 (0.6%) out of 1,702 stool samples examined. Infection rate was higher among aged males. Most of the subjects with *Giardia* organisms had no history of traveling overseas except 2 cases. Although source of infection was not determined, most of the positive subjects supposed to be infected within the community.

Key word: *Giardia*, Giardiasis, Diarrhea, Soft feces, Advanced age group

INTRODUCTION

Giardiasis is one of the common intestinal protozoan infections. This disease is prevailing in developing countries (Tompson *et al.*, 1993). Even in developed countries, infections with *Giardia* spp. have been reported from day-care centers (Pickering *et al.*, 1981). Food-borne transmission (Peterson *et al.*, 1988) and water-borne transmission (Moorehead *et al.*, 1990) have also been reported. Reflecting recent advancement in international interchange and traveling abroad, imported cases have been increasing in Japan. In addition, a close association between sexually-transmitted diseases and protozoan infection has been focused (Takeuchi and Miyahira, 1989). To date, several data on prevalence of giardiasis diagnosed by stool examination have been reported by health screening and from admitted patients in different 2 hospitals in metropolitan area and Tokai region in Japan (Kado *et al.*, 1986; Ichizawa *et al.*, 1990). However, a few cases regarding the disease have been reported from Shikoku region, especially from Kochi Prefecture, which is different from other metropolitan area in socio-economics as well as population structure including life styles of the people.

SUBJECTS AND METHODS

Study subjects

Stool samples were collected from 1,702 subjects of 0-94 years of age who visited the Kochi Medical School Hospital either as admitted or outpatients from April 1996 to January 1998. These subjects included 950 males and 752 females who had either history of diarrhea or passage of soft stool.

Stool examination

Fecal specimens were examined microscopically by the direct smear method for trophozoites or cysts of *Giardia* spp. To confirm *Giardia*-positive cases, they were processed by Wright's solution or modified Kohn's one-step staining (Gleason and Healy, 1965). Smears from diarrhea samples were also stained with modified Ziehl-Neelsen carbol-fuchsin to detect *Cryptosporidium* oocysts (Henriksen and Pohlenz, 1981).

Examination of blood

Blood was tested for peripheral blood cell counts, immunoglobulin assay and biochemical analysis before and after the treatment in patients with *Giardia* parasites. Particle agglutination tests (Fujirebio, Tokyo, Japan) were performed for the measurement of serum antibody against human T-cell lymphotropic virus-type

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Table 1 Age and sex-distribution of subjects examined for *Giardia* infection

Age (years)	Total examined	Male		Female	
		Negative	Positive (%)	Negative	Positive (%)
≤10	87	55	0 (0.0)	32	0 (0.0)
11-20	70	31	0 (0.0)	39	0 (0.0)
21-30	73	34	0 (0.0)	39	0 (0.0)
31-40	79	37	0 (0.0)	42	0 (0.0)
41-50	188	90	0 (0.0)	98	0 (0.0)
51-60	257	133	2 (1.5)	121	1 (0.8)
61-70	507	308	5 (1.6)	194	0 (0.0)
71-80	361	214	1 (0.5)	146	0 (0.0)
≥81	80	39	1 (2.6)	40	0 (0.0)
Total	1,702	941	9 (1.0)	751	1 (0.1)

I (HTLV-I).

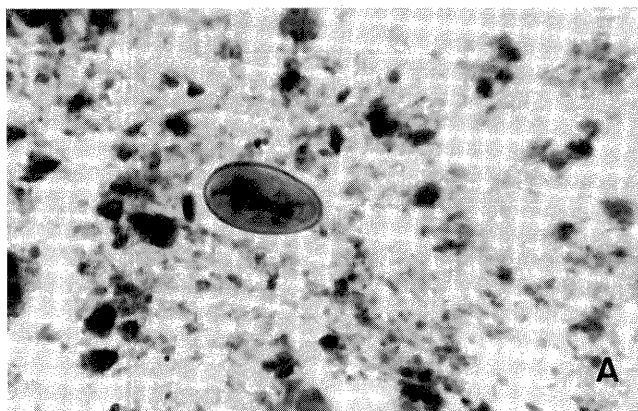
Statistical analysis

Contingency table analysis was used to determine the probability of significant differences between the sex and age groups. Paired *t* test was applied to evaluate hematological data. A value (*P*) of less than 0.05 was considered significant.

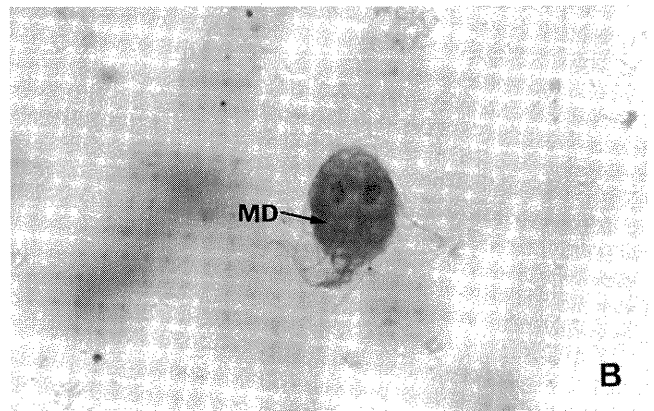
RESULTS AND DISCUSSION

The prevalence rate of positive cases for *Giardia* organisms is shown by sex and age in Table 1. Out of 1,702 subjects examined in the present study, stool samples from 10 subjects (0.6%) consisting of 9 males and 1 female were positive for cysts of *Giardia* by microscopic examination. In most of the cases, only cysts of *Giardia* spp. were found in fecal samples. Trophozoites were observed in fecal samples from 2 cases and bile sample from one patient. The species of the genus *Giardia* have

been reported to be divided into three groups (*G. agilis* group, *G. muris* group and *G. duodenalis* (= *G. intestinalis* = *G. lamblia*) group) differing in the shape and position of the median bodies, the shape of the cell, and the relative length of the adhesive disk (Kulda and Nohýnková, 1978; Tompson *et al.*, 1993). The morphology of the trophozoites observed was pyriform in shape (body length; $13.6 \pm 1.3 \mu\text{m}$, adhesive disk length; $6.3 \pm 0.6 \mu\text{m}$, $n=30$) and the median body was situated approximately across the body (Fig 1). Based on these morphological features, the organisms were identified as trophozoites of *Giardia lamblia*. To date, it is not possible to distinguish morphologically the cyst of *G. lamblia* from that of other *Giardia* spp. Therefore, it should be necessary to develop a molecular genetic analysis, using PCR, in the future to identify the species of the cysts in the fecal samples. In this study, *Cryptosporidium* and other protozoa were not found in diarrheal stool samples examined. The characteristic



(A)



(B)

Figure 1 (A) Cyst of *Giardia* stained with modified Kohn's one-step staining ($\times 1,000$). (B) Trophozoite of *G. lamblia* Stained with Wright's solution. The median body (MD) is situated approximately across the body ($\times 1,000$).

Table 2 Summary of the subjects with *Giardia* organisms

No.	Age (years)	Sex	Travel History*	Detection of trophozoites	Symptoms †	Clinical History ‡	Drug §
1.	69	M	SEA	—	Abdominal pain, ailment	Cancer (tongue)	N
2.	72	M	NA	—	NA	Cardiac disease, Diabetes mellitus	NA
3.	61	M	N	—	Diarrhea, Abdominal ailment	Cancer (lung)	CD
4.	68	M	N	+	N	Cancer (lung)	N
5.	60	M	N	+	Diarrhea, Abdominal ailment	Cerebral infarction	N
6.	67	M	NA	—	NA	Cancer (mesopharynx), Pneumonia	CD
7.	60	F	N	—	N	Plasmacytoma	CD
8.	81	M	Eur	—	N	Diabetes mellitus, Gout	N
9.	70	M	N	—	N	Cardiac disease	N
10.	58	M	NA	—	N	Cardiac disease	N

*Eur=Europe; N=none; NA=no answer; SEA=Southeast Asia.

† Main symptoms relating to giardiasis

‡ Main history

§ Administration of immunosuppressive drug or carcinostatic drugs (CD)

feature of the present study is that *Giardia*-positive cases were distributed in advanced age group (>51 years old). This tendency is not consistent with age distribution in developing countries (Gilman *et al.*, 1985). The prevalence rate was comparable to those reported from Fukuoka, Tokyo and Shizuoka in Japan by Mako (1982), Kado *et al.* (1986) and Ichizawa *et al.* (1990) respectively. Occupation of the subjects with *Giardia* parasites was different.

A few subjects had symptoms related to *Giardia* infection (Table 2). Most of the subjects had history of malignant neoplasm and cardiac diseases, and some had been taking carcinostatic drugs. None of the subjects was infected with HTLV-I, although the disease is endemic in Kochi (Taguchi *et al.*, 1983). One out of 8 subjects with *Giardia* parasites was resistant to the treatment with metronidazole. It has been reported that drug resistance in giardiasis is related with agammaglobulinemia, intestinal hyposecretion of IgA, hypoacidity and etc. (Wolf 1975; Akahori *et al.*, 1994). In the present metronidazole-resistant case, however, a serum immunoglobulin level was normal. It has been suggested that host's cellular cytotoxicity for the parasite is involved in chronic giardiasis (Smith *et al.*, 1982). Monocyte counts in peripheral blood of several subjects positive for *Giardia* decreased after treatment with metronidazole (Fig 2). Monocyte-derived macrophages have been reported to have capacity to ingest *Giardia* trophozoites and to kill intracellular parasites (Hill and Pearson, 1987), although it remains to be solved that monocytes are important effector cell *in vivo*.

Fecal examination was done of all the member of a family living with one parasitologically-confirmed subject. None of the family members had been found

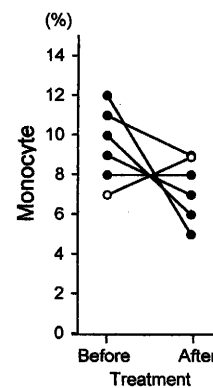


Figure 2 Monocyte rates in peripheral blood from subjects with *Giardia* organisms before and after treatment with metronidazole. Statistical analysis excluded data of which one patient (open circle) persisted in passing cysts of *Giardia* in his stool after treatment.

infected with *Giardia*. Most of the parasite-positive subjects had no pet animal. At least some isolates of *Giardia* have a wide range of host susceptibility. Therefore, it is possible that humans and a variety of animals naturally share the parasite (Baker and Gerschwin, 1987; Collins *et al.*, 1987; Thompson 1992). Two subjects positive for the organism went abroad within last several years. Most of the positive cases in the present study were thought to be infected within their community, but the source of infection was not determined.

In conclusion, we showed the higher prevalence of *Giardia* spp. infection in advanced age group, when stool samples from subjects of 0 to 94 years of age were examined. Such a tendency might be caused by an immunological condition of the host subjects such as immunodepression by aging. Nowadays, in Japan, the

ratio of advanced age group has been increasing especially in Kochi Prefecture. Therefore, more attention should be given to such parasitic infections at laboratory stool examinations, in order to eliminate cyst carriers as potential source of the infection.

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