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Cryptosporidium Sp. Findings and Its Symptomatology among Immunocompromised Patients

Dewi Masyithah Darlan[1*], Muhammad Fakhrur Rozi[2], Yunilda Andriyani[1], Hemma Yulfi[1], Restuti Hidayani Saragih[3], Nerdy Nerdy[4]

[1]Department of Parasitology, Faculty of Medicine, Universitas Sumatera Utara, Padang Bulan, Medan Baru, Medan, Sumatera Utara, Indonesia; [2]Faculty of Medicine, Universitas Sumatera Utara, Padang Bulan, Medan Baru, Medan, Sumatera Utara, Indonesia; [3]Division of Tropical and Infectious Disease, Department of Internal Medicine, Faculty of Medicine, Universitas Sumatera Utara, Padang Bulan, Medan, Sumatera Utara, Indonesia; [4]Department of Pharmacy, Academy of Pharmacy Yayasan Tenaga Pembangunan Arjuna, Pintubosi, Laguboti, Toba Samosir, Sumatera Utara, Indonesia

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***Correspondence:** Dewi Masyithah Darlan. Department of Parasitology, Faculty of Medicine, Universitas Sumatera Utara, Padang Bulan, Medan Baru, Medan, Sumatera Utara, Indonesia. E-mail: nerdy190690@gmail.com

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Abstract

BACKGROUND: *Cryptosporidium sp.* is an apicomplexan protozoa, and it is related to an immunocompromised state. As it develops diverse clinical manifestations, mild to life-threatening conditions, administration of anti-parasitic medication and its management remain problematic.

AIM: The study aimed to provide Cryptosporidiosis symptomatology and its prevalence among HIV-infected patients in a tertiary referral hospital, Haji Adam Malik General Hospital, Medan, Indonesia.

MATERIAL AND METHODS: Symptomatology was noted using short-questionnaire, and laboratory findings were obtained from the hospital medical record registry on the same day of admission. We enrolled 24 patients were suffered from HIV infection for a certain period and more than one-week diarrhoea including 18 males and 6 females. Routine faeces examination using wet mount, Kinyoun-gabet, and trichrome staining was performed for all samples in Parasitology Department, Faculty of Medicine, Universitas Sumatera Utara, Medan, Indonesia. Numerical data were evaluated using the Mann-Whitney test while Fisher Exact test was used to determine any association between categorical variables.

RESULTS: Our study found that 8 of 24 patients were positive with *Cryptosporidium sp.* while its symptomatology including abdominal cramp (66.7%), nausea and vomiting (70.8%), and fever (62.5%) is prevalent from our study. We obtained significant association between CD4 cell count (p = 0.006), diarrhea duration (p = 0.007), abdominal pain (p = 0.005), and nausea and vomiting (p = 0.021) with cryptosporidiosis.

CONCLUSION: High consideration of several symptoms related to cryptosporidiosis leads a clinician to initiate prompt management particularly in a high-risk population.

Introduction

Cryptosporidium sp. first appearance was noted in 1976 when the organism infected a three-year-old child with self-limiting enterocolitis while in early 1980s Cryptosporidium sp. had been linked to the immunocompromised population [1]. *Cryptosporidium hominis* and *Cryptosporidium parvum* are commonly associated with human infection. Ingestion of fully and chlorine resistant sporulated oocyst also initiate the infection in human. After the oocyst ingestion, upper small bowel will become the milieu for infection, and it develops into the further stage, as the sporozoite penetrates the enterocyte and transforms into the mature form [2]. The global burden for cryptosporidiosis was noted from several reports in Africa and Asia, 11.3% in Turkey [3], 6% in Iran [4], and the highest prevalence found in Uganda as much as 73.6% [5]. Indonesia

data prevalence of the infection is available based on two studies ranging from 4.9-52.5% [6], [7]. Most studies revealed that the prevalence variation of *Cryptosporidium sp.* among HIV-infected population occurred since the limitation of using proper diagnostic method particularly in developing regions is established [8].

The infection could produce broad clinical manifestations ranging from mild to life-threatening symptoms depends on the presence of comorbidities particularly HIV/AIDS status, malignancy, patients undergoing dialysis, and organ transplantation [9]. *Cryptosporidium sp.* as the most common species found in the HIV population and it is linked to higher mortality among population. Diarrhoea associated with cryptosporidiosis is watery or mucoid diarrhoea that persists ranging from acute to chronic form which caused several clinical implications to the patients including severe dehydration, abdominal pain, nausea and vomiting, fever, malnutrition, and significant weight loss [10]. Medication for cryptosporidiosis still becomes a problematic issue as its effectivity among the certain population is questionable while U.S Food and Drug Administration (FDA) only approved nitazoxanide which does not effectively eradicate the organism in immunocompromised hosts [11].

There has been no study related to symptomatology and cryptosporidiosis in Indonesia recently. Our study aimed to provide the prevalence of cryptosporidiosis among the HIV population and its symptomatology in a single institution, tertiary referral centre. Assessment and evaluation may be used to determine the likelihood that patients with certain symptomatology have *Cryptosporidium sp.* infection.

Methods

Study location and population

We enrolled in a cross-sectional study conducted in one tertiary referral centre, Haji Adam Malik General Hospital, Medan, Indonesia. The study included 18 males and 6 females who had met the inclusion criteria, for instance: HIV positive patients, suffered from more than one-week diarrhoea and admitted to the outpatient clinic or hospitalisation ward. WHO defines diarrhoea as three or more loose stool per day for 72 hours (acute for no more than 13 days; chronic or persistent diarrhoea is defined as diarrhoea for 14 days or more). Medical record registry was assessed to fulfil several variables related to demographic data (gender and age), and laboratory results including CD4+ cell count, haemoglobin, hematocrit, leukocyte, thrombocyte, urea, creatinine, and glucose levels. The laboratory results were obtained from Haji Adam Malik medical record registry on the same day of their admission to the hospital. A short questionnaire was also used to determine symptomatology of all participants consisting of three-panel question about the presence of abdominal pain, nausea and vomiting, and fever; the physical examination was performed to ascertain the symptomatology.

Parasitological examination

All participants had given their approval to include in the study. On the day of admission, a small faecal sample container was given to the patients. On the following day, sample collection was performed by our laboratory staff; no preservation was applied to samples. In the same day of sample collection, the microscopic examination was carried out in the Parasitology Department, Faculty of Medicine, Universitas Sumatera Utara, Medan, Indonesia. A battery of examination was conducted to determine the presence of Cryptosporidium sp., and other pathogenic parasites including routine faeces examination using wet mount, Kinyoun-gabet, and trichrome staining. As a matter of limitation in the study, bacterial culture and viral marker examination were not screened. The study had been evaluated by Health Research Ethical Committee of Faculty of Medicine, Universitas Sumatera Utara and obtained their approval.

Statistics and study approval

The research form and questionnaire were collected then transformed into one table in Microsoft Excel. Statistical Package for Social Sciences 21 (SPSS Inc. version 21) in univariate fashioned using Fisher Exact test was used to determine any significant presence relationship between several variables and Cryptosporidiosis. Furthermore, numeric data were evaluated using the non-parametric Mann-Whitney test to evaluate the mean difference between positive and negative groups of cryptosporidiosis including age, CD4+ cell count, diarrhoea duration and several laboratory results.

Results

We proved that 8 of 24 patients were positive for Cryptosporidium sp. and one patient was positive with double infection, *Cryptosporidium sp.* and *Blastocystis hominis*. The demographic data were depicted in Table 1. One-week diarrhoea was commonly found in our study while there was one patient who had suffered from 24-week-diarrhoea. Symptomatology among patients was also assessed; it revealed that abdominal pain (66.7%), nausea and vomiting (70.8%), and fever (62.5%) were prevalent

among participants. Laboratory results baseline also listed in Table 1.

The symptomatology including abdominal pain, nausea and vomiting, and fever was evaluated using short questionnaire while abdominal pain and nausea and vomiting had a significant association with the infection, p = 0.005 PR 21.00 95% CI 2.372-185.93 and p = 0.021 PR 0.086 95% CI 0.011-0.673 respectively. Abdominal pain was complained by 6 of 8 cryptosporidiosis patients. Nevertheless, we proved descriptively that most of the patients who complained about nausea and vomiting were demonstrated as negative for *Cryptosporidium sp.* Several variables, then, was also statistically evaluated using Mann-Whitney testing since the data was not normally distributed. We obtained significant association between CD4+ cell count (p = 0.006), diarrhea duration (p = 0.007), and leukocyte (p = 0.038) with cryptosporidiosis.

Table 1: Several variables among cryptosporidiosis patients

Characteristics	Cryptosporidiosis (Mean ± SD)		p-value
	Positive (8/33.3%)	Negative (16/66.7%)	
Age (year)	36.50 ± 7.28	42.93 ± 12.10	0.238
Gender (Male/Female)	5/3	13/3	0.621
CD4+ cell count (/µL)	87.37 ± 141.16	319.50 ± 160.39	0.006*
Diarrhea duration (weeks)	7.25 ± 7.34	1.81 ± 1.10	0.007*
Laboratory results			
Hemoglobin (gr/dL)	8.98 ± 1.42	10.64 ± 1.88	0.070
Thrombocyte (cell/ µL)	195,250.00 ± 81,32	229,537.50 ± 117,216.30	0.490
Hematokrit (%)	37.10 ± 11.83	35.37 ± 7.09	0.834
Leukocyte (cell/µL)	6,428.75 ± 2,475.04	9,238.12 ± 3,220.81	0.038*
Glucose levels (mg/dL)	86.12 ± 15.19	117.18 ± 76.95	0.320
Creatinin (mg/dL)	0.88 ± 0.60	2.04 ± 2.33	0.291
Ureum (mg/dL)	32.40 ± 21.51	68.49 ± 73.15	0.320
Symptomatology (Yes/No)			
Abdominal pain	6/2	2/14	0.005*
Nausea and vomiting	3/5	14/2	0.021*
Fever	5/3	10/6	1.000

*, statistically significant.

Discussion

As part of the neglected tropical disease, cryptosporidiosis still becomes problematic since it can produce several symptoms ranging from mild, self-limiting disease to severe dehydration or death [12]. Also, *Cryptosporidium sp.* is categorised as a highly infectious organism, infectious dose until it can initiate infection as low as 10 oocysts [13]. Immunocompromised status particularly HIV/AIDS infection also had been linked to the infection and its presence become a leading causative agent can produce the debilitating condition among the population. Inadequacy of the diagnostic method of Cryptosporidium sp. also challenges the findings of the organism [14].

Immunologic response of *Cryptosporidium sp.* invasion is emphasised from literature, the elevation of certain cytokines such as interferon-gamma (IFN-Y), interleukin-1β (IL-1β), tumour necrosis factor-alpha (TNF-α), interleukin-10 (IL-10) and transforming growth factor-β (TGF- β), is evident [15]. Intestinal epithelial cells (IECs) detect *Cryptosporidium sp.* and initiate the recognition of the organism to CD4+ cell via Major Histocompatibility Complex-II (MHC-II) [16]. As HIV destruct CD4+ cell, cell-mediated immunity arm among HIV-infected individuals will severely impair resulting in ineffective eradication of the organisms [17], [18]. Our study proved a significant difference in CD4+ cell count between two groups of patients, infected- and uninfected-group (p = 0.006). Cryptosporidiosis group had CD4+ cell count which was significantly lower than the negative group. Furthermore, the likelihood of getting the infection increases after CD4+ cell count falls to 50 cell/mm3 [19], [20].

Morphologic changes including loss of microvilli resulting in disruption of several enzymes involved in the digestion process are crucial for developing symptoms among infected-individual [21]. Cryptosporidiosis patients mostly complained of watery diarrhoea, abdominal pain, anorexia, flatulence, nausea and vomiting, and mild fever. Significant association was obtained from our study particularly abdominal pain was related to cryptosporidiosis (p = 0.005) while nausea and vomiting was significantly associated with cryptosporidiosis in inversion manner (p = 0.021) since our study showed most patients complained the symptoms were negative for *Cryptosporidium sp.* (infected-patients complained nausea and vomiting versus uninfected-patients; 37.5% versus 87.5%). There are few published articles linked *Cryptosporidium sp.* with the presence of certain symptoms among the HIV population in Indonesia. Notwithstanding, several studies revealed the prevalence and symptoms produced during the infection, for instance, Wanyiri et al., [22] conducted a study in Kenya found 56 of 164 (34%) of HIV/AIDS patients were positive for *Cryptosporidium sp.* A significant association between abdominal pain and vomiting was also evident in the study. In Iran, Izadi et al., [23] also revealed similar results that two common symptomatology commonly found in cryptosporidiosis patients including abdominal pain and nausea and vomiting had a significant association with the infection.

Association between chronicity and cryptosporidiosis among the HIV population stated from the literature [24], [25]. While our study obtained a significant difference between two groups, the mean of diarrhoea duration among infected group versus uninfected group was 7.25 versus 1.81 (p = 0.007). The condition may be clearly explained since the mainstay of therapy among HIV/AIDS population is the restoration of immune function. Immune reconstitution shortly occurred after effective administration of combination antiretroviral therapy has been associated with parasitic clearance. Duration of symptoms especially diarrhoea is directly linked to the immune response in each [26].

Also, symptomatic management including

replacement of fluids and electrolytes is essential to prevent further complications. Furthermore, administration of any anti-parasitic medication remains debatable [27].

In conclusion, the study has revealed several significant results by using a smaller sample size, yet it becomes the limitation of the study. Also, the evidence also has brought several clinical significances among cryptosporidiosis patients and the symptomatology. Therefore, the combination of HIV/AIDS positive status, diarrhoea, and abdominal pain increase the likelihood of a patient having the infection. The infection remains neglected not only in the general population but also among HIV/AIDS patients. The physician should increase awareness of the infection among HIV/AIDS patients since the infection and diarrhoea shown to be an independent predictor of mortality in the population.

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