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Cryptosporidium sp. and Blastocystishominis Findings: A cross-sectional Study among Healthy Versus Immunocompromised Individuals

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Abstract---

Background: *Cryptosporidium sp. and Blastocystishominis are widely recognized as opportunistic intestinal parasites among the general population, particularly immunocompromised individuals producing acute or chronic diarrhea. Nevertheless, B.hominis still become dubious as its pathogenesis has not yet fully elucidated. Objective: The study aims to prove the classical findings of the theoretical background, whether or not Cryptosporidium sp. and B.hominis associated with immunocompromised conditions.* ***Materials and methods:*** *The cross-sectional study enrolled 52 participants then divided into two groups: HIV positive (29 patients) and healthy individuals (23 patients) who involuntarily provide their feces for microscopic parasitic examination in Department of Parasitology, Faculty of Medicine, Universitas Sumatera Utara, Medan, Indonesia. All data were presented in bivariate mode to undergo further statistical analysis using chi-square test.* ***Results:*** *There are descriptive findings proved that B.hominis found mostly among HIV positive (7 samples) while only two positive samples in the immunocompetent group, yet no significant association between the infection and HIV status has been obtained (p= 0.144; OR 0.299 95%CI 0.056- 1.608). Similarly, it was also not statistically proven that Cryptosporidium sp. infection and HIV status havea significant relationship (p= 0.197; OR 4.20 95%CI 0.407- 43.374).* ***Conclusions:*** *In addition to its uncertain pathogenesis, B.hominis still become neglected parasitic infection among HIV population while Cryptosporidium sp. confounds by other factors, mainly CD4 count of each individual for the infection to occur.*

Keywords--- *Immunocompromised, HIV, Intestinal Protozoa, Parasite.*

I. INTRODUCTION

Cryptosporidium sp. and *Blastocystishominis* can produce infection among animals and humans who susceptible to diseases. There is also a wide range of pathogenicity and virulence of the organisms highly depends on host susceptibility and organism strain per se.[1]*Cryptosporidium* was firstly discovered in the 20th century, *Cryptosporidium muris* and *C.parvum* as its first identified species. In the early 1980s, an outbreak of *Cryptosporidium* occurred among AIDS patients producing severe watery diarrhea that finally led to higher

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mortalities, from then on Cryptosporidiosis has been described as AIDS-associated infection. The infection is also not limited to immunocompromised population; *Cryptosporidium sp.* can also lead an infection among immunocompetent individuals. Nevertheless, low CD4 cell count (<50) has been regarded as a significant risk factor for initiating the infection.[2] As a zoonotic protozoal disease, its species was called after the name of infected-species, such as *Cryptosporidium meleagridis* for birds or human, *C.parvum* for cattle and human, *C. canis* for dog, *C.felis* for cat, *C.muris* for rodent, *C.andersoni* for cattle, and *C.hominis* which causes infection for 90% of human cryptosporidiosis cases. *Cryptosporidium sp.* life cycle only occurs in one host species after the ingestion of small oocysts for encystation in small intestine, starting from merogony, gametogony, zygote production, lastly yielding sturdy oocysts to transmit the infection.[3] In several epidemiologic surveys, it was found that higher prevalence of cryptosporidiosis in developing countries, 10.8% in Iran, 10-37% in Nigeria, and the fact 77% among Bangladeshi children.[4]

Meanwhile, the pathogenic characteristic of *Blastocystishominis* has been doubted for several decades and becoming neglected parasitic infection until today. As a pathogen causing gastrointestinal symptoms, it also remains questionable. There are several aspects including nomenclature, life cycle and transmission, precise clinical symptomatology, and its management needs to be discussed subsequently, several studies are also tried to plunge into the dubious description of organisms.[5]Alexeieff firstly introduced *Blastocystis sp.*as a different organism and replaced *B.enterocola* to Blastocystis, yet Brumptnewly proposed *B.hominis*, a term used this time. The organism has been re-classifiedinto a separate group as stramenopile, a heterotrophic-photosynthetic protist, and the only organism in stramenopile that could lead to human infection.[6] Similar to *Cryptosporidium sp*., *B.hominis* are also widely found among animals and humans, but the global prevalence of the infection is unknown. There have been several reports of prevalence in most parts of the world, such as 58 % of infection amongthe Indonesia population.[7]There is also an emerging problem relating to the high-concern of *B.hominis* infection for human health; it is the rising prevalence of HIV infection among the general population. Immunocompromised conditions induce severe form of *B.hominis* infection and could lead to higher mortality in the targeted population. Therefore, there is evidence that the infection could associate with CD4+ cell count or immune status. The infection is not only associated with HIV positive patients but also individuals with malignancy or other types of impaired cellular immune function. In Turkey, *B.hominis* is prevalent among cancer patients while, in other parts of the world, *B.hominis* is highly linked with close animal contactor as zoonotic infection. In Nepal, it is demonstrated that no relationship between *Blastocystis sp.* and traveler's diarrhea has been obtained, but descriptively it was found that 25%-75% of Blastocystis positive patients is previously positive for history of foreign travel.[8,9]

Therefore, there is a direct correlation of CD4+ cell count as well as the immunocompromised condition and the infection of *Cryptosporidium* and *B.hominis* based on essential theoretical background. Consequently, the study aimed to investigateclassical findings of the infection among two groups (immunocompetent and immunocompromised which represented by the HIV-infected population.

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II. METHODOLOGY / MATERIALS

There was a brief oral explanation to all participants who gave consent for the enrollment of the study as well as filling the short questionnaire for any previous or existing illnesses. In the setting of Tertiary Referral hospital/ Haji Adam Malik General Hospital, the study obtained 29 participants of HIV patients, healthy individuals (23 participants) were acquired from primary care setting of Padang Bulan, Medan, Indonesia. One small plastic fecal container labeled with the individual's name was given for the fecal sample examination in the Department of Parasitology, Faculty of Medicine, Universitas Sumatera Utara, Medan, Indonesia, between August and September 2019. The study used the consecutive technique with period-limited sampling (spans for three weeks of sampling), it includes all the fitted-samples to the inclusion criteria, such as confirmed-HIV status with the latest CD4+ cell count for HIV group while no recent or previous history of immunocompromised disease for healthy group.The parasitological examination for *Cryptosporidium sp.* used modified acid-fast stain or Kinyoun-gabet staining while Lugol and trichrome staining for *Blastocystishominis* infections.All data were doubled-checked in Microsoft Excel before the final analysis using Statistical Package for Social Sciences 21 (SPSS Inc. version 21). Subsequently, the relationship between the organism infection and HIV status was demonstrated using p-value < 0.05, which extracted from the chi-square test and presented the data into a single table. The study has also obtained approval from the Medical Ethics Committee, Faculty of Medicine, Universitas Sumatera Utara, Medan, Indonesia.

III. RESULTS AND DISCUSSIONS

The study involved 42 males and 10 females who had given their consent and fulfilled the inclusion criteria. In further separation, there are 29 HIV participants and 23 healthy individuals, subsequently underwent parasitological examination which revealed there are four microscopically-confirmed cryptosporidiosis patients, one in HIV group and three in healthy individuals. Additionally, there were also nine positive *Blastocystishominis* patients with higher prevalence demonstrated in the HIV group (seven patients) and two patients in healthy individuals.Nevertheless, the organism infection did not have any significant relationship with the HIV infection in the study, p=0.197 and 0.144, for *Cryptosporidium sp.* and *Blastocystishominis* respectively. The HIV participants routinely controlled for the CD4 count+ and evaluated the response for therapy, particularly administration of antiretroviral (ARV) medication. The symptomatology of the patients was not demonstrated since the study has no aims to determine the symptoms of the infection but the relationship between the infection and HIV infection. However, CD4+ cell count data were noted at the preliminary stage of the study.

Table 1: Demographic characteristics and relationship of the infection and immunocompromised condition.

Characteristics	Immunocompromised N (%)		OR (95% CI)	p-value
	Yes	No		
Sex				
Male	23 (54.8)	19 (45.2)	0.807	0.764
Female	6 (60.0)	4 (40.0)	(0.198-3.284)	
Age (year old on average)	38.31	31.35	-	-
Cryptosporidium sp.				
Yes	1 (25.0)	3 (75.0)	4.200	0.197
No	28 (58.3)	20 (41.7)	(0.407-43.374)	
Blastocystishominis				
Yes	7 (77.8)	2 (22.2)	0.299	0.144
No	22 (51.2)	21 (48.8)	(0.056-1.608)	

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AIDS has become a burden worldwide because it induces higher morbidities relating to the appearance of other infections and diseases. Intestinal parasitic infection arises to be the predominant causative agent for diarrhoeic patients in the HIV population, mainly intracellular protozoa such as *Isospora belli, Cryptosporidium parvum,* and *Cylospora sp.* The connecting line between the emergence of the parasitic infection and immunocompromised condition has been previously studied.[10]The immune clearance of intracellular parasites uses cellular immune function to eradicate the infection in which HIV has impaired its function. Therefore, CD4+ cell count could be used as predictive tools of the infection and primarily linked with the incidence as well as the severity of the infection.[11]

Blastocystishominis are widely distributed worldwide, with a higher prevalence found among immunocompromised patients and a group of people who has frequent animal contact.[12] The initiation of infection occurs after the oocyst ingestion in contaminated-water; furthermore, it produces significant symptoms following individuals' immune status as well as the burden of organisms in one period of infection.[13] Non-specific symptoms, including bloating, cramping, severely watery diarrhea, or vomiting could accompany the infection.[14] Interaction effect of CD4+ cell count and other risk factors have been reportedly proven in a study conducted by Zhang et al., it revealed that raising animals and drinking un-boiled water as the significant risk factors for Blastocytosis in HIV patients while CD4+ cell count per se was responsible for the prevalence and detection rate of infection compared to the subjects with higher CD4+ cell count and lower HIV viral load.[15]

Cryptosporidiosis has commonly produce infection in four different types of population, such as children in developing countries, traveler's diarrhea to developing countries, immunocompromised population, and outbreaks in developed regions. After 3-12 days of the incubation period, the symptoms of the infection appear as if the other parasitic infection including giardiasis, isosporiasis, and cyclosporiasis so the diarrheic patients need reassurance for parasitic examination under microscopemainly if the individuals abide with the risk factors of the infection.[16] The symptoms span from mild until a severe form of infection including fever, abdominal pain, anorexia, malaise, flatulence, vomiting, and mainly diarrhea.[17]*Cryptosporidium sp*. has also been notable as AIDS-associated infection from literature and related tolower CD4+ cell count for its occurrence.[18]

Oocyst will spread out across the mucosal epithelium and Peyer's patch M cells to be inoculated intothe surrounding tissue. Intestinal epithelial cells (IECs) contained toll-like receptors (TLRs) and intracellular nod-like receptors (NLRs) which recognized the organisms as the new threat; subsequently, it will activate resident macrophage and finally transform into antigen-presenting cells (APCs) via Major Histocompatibility Complex (MHC)-II to eradicate the infection. The presentation involves CD4+, which turns into activated- T cells that could lead to further activation of cytokines and adhesion molecules, and it signs the activation of acquired immune system.[18]There have been significant findings of CXCL-10, and CXCL-8 become the pivotal chemokine secreted during the infection period.Activated T-cell has undoubtedly secreted IFN-ϒ as the primary cytokine for a parasitic immune response during the acquired immune arm activation.[19]The secretion of several cytokines relates to cellular immune function; therefore,the insufficient immune response to *Cryptosporidium sp.* is commonly found among HIV individuals. The inadequacy of organism clearance has appeared as the problems emerge among the HIV population as the severe form of the disease frequently occurred producing debilitating symptoms afterward.[20,21]

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IV. CONCLUSION

Cryptosporidium sp. and *Blastocystishominis* are the two organisms which commonly recognized as AIDS-associated infection. Nevertheless, because of unclear pathogenesis, *B.hominis* has been neglected and misinterpreted as other causes of infection, particularly in immunocompetent people. In the study, no significant relationship between the infection and HIV status because it might be affected by some confounding factors, particularly HIV infection duration, as well as CD4 cell count. Therefore, multivariate analysis involving a larger sample size needs to be performed to establish a relationship of infection in the general population.

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