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Prevalence of Cryptosporidiosis among Cancer Patients in Sulaimani Province/Iraq

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Abstract: *The intestinal protozoan parasites are common pathogens of the gastrointestinal tract (GIT) and diarrhea among immunocompromised patients. Entamoeba histolytica, Giardia lamblia, and opportunistic Cryptosporidium spp. are considered as the most types of intestinal parasites. Cryptosporidium is an obligate intracellular protozoan microorganism that causes gastrointestinal infection (cryptosporidiosis), which is an opportunistic intestinal parasite among cancer and immunocompromised patients, it is a major cause of life-threating diarrhea among immunosuppressive patients. This study included 365 cases (300 cancer patients and 65 non cancer individuals). The Modified Ziehl-Neelsen (MZN) staining and crypto-strip (Immunochromatographic Assay) techniques were followed for Cryptosporidium oocyst's detection in human feces. Prevalence of Cryptosporidium species among cancer patients was 17% while among non-cancer patients was 3%. The gender and residency have no significant effect on cryptosporidiosis, the significant differences were observed between cryptosporidiosis cases and stool consistency. However, a relatively higher rate of Cryptosporidiosis was observed among old age (≥ 51 years) with cancer undergoing chemotherapy. This finding concludes that Cryptosporidium spp. are considered as a common diarrheal causative agent among cancer patients and need more care and management.*

Keywords: *Cryptosporidiosis, Cancer, Modified Ziehl -Neelsen, Diarrhea, AFS, Immunocompromised.*

Introduction

Intestinal protozoan infection remains an important community health concern due to the increased rate of its prevalence and nutritional consequences. Intestinal protozoan parasites are common pathogens that cause gastrointestinal tract infection (GIT) and diarrhea among children and immunocompromised patients [1], the common types of intestinal protozoa are *Giardia lamblia*, *Entamoeba histolytica*, *E. coli Blastocyst hominins,* and *Cryptosporidium spp*. [2]. Generally, they have a dual effect on the humans directly they affect the lining of the intestinal lumen and they will damage or alternate the cell surface and indirectly they lead to malabsorption and malnutrition then affect the immune system [3].

Cryptosporidium spp. are an opportunistic protozoan parasite responsible of acute gastroenteritis and moderate to severe diarrhea among children and immunocompromised patients. The *Cryptosporidium spp.* infection is life-threatening for immunocompromised patients [4]. This parasite is an opportunistic intestinal parasite that may be infecting humans and animals [5], it can cause infection among all age groups, particularly among children and elderly peoples [6], Cryptosporidium has a wide range of hosts with a low host-specific and cosmopolitan distribution.

The life cycle of Cryptosporidium is simple and it will continue in one host, the Infection is initiated by the ingestion of an environmentally resistant Oocyst by the hosts [7]. The sporozoites can invade the intestinal cells after excystation (excite the sporozoite from the Oocyst), the rupture of the oocyst wall allows releasing four infectious sporozoites from each the mature Oocyst [8].

Oocyst of Cryptosporidium is the infective stage and resistant to the most chemical disinfectants particularly chlorination even at high doses but sensitive to a high dose of UV [9]. While it also could be killed by ammonia, formol-

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saline, temperatures lower than 0 °C and higher than 65 °C for 30 min, and full-strength commercial bleach. The electronic microscopic image of the Oocyst shows the oocyst wall compost from 2 or 3 distinct layers [10].

Cryptosporidium oocyst is either thick wall or thin wall oocyst, the thin-walled oocyst when separated from epithelial tissue can cause autoinfection for the same host and the cycle starts again, while thick-walled oocyst which is resistant to environmental condition causes infection in another host [9].

The human will get the infection through contaminated water or foods with the Oocyst of Cryptosporidium [6]. The molecular techniques allow researchers to identify at least 16 species of Cryptosporidium, two of them; *Cryptosporidium hominins* and *Cryptosporidium parvum* are the main causative species of human cryptosporidiosis [11].

This parasite has global distribution with a high potential ability to infect humans and other invertebrates [12]. Also in the developing countries, about 1% to 10% of the populations were suffering from Cryptosporidium infection or *C. hominis*, in which children and toddlers with age (1 to 9 years old) were the most affected age group [13]. Cryptosporidiosis in developing countries causes intestinal mal-absorption and further affects especially malnourished children then has an indirect effect on the immune system and immunoglobulin production [14]. Furthermore, due to the common interest and community health concern cryptosporidiosis started from the last two decades, when more than 400,000 people in Milwaukee, United State of America were infected by *Cryptosporidium hominis*, this is happened because of fecal contamination in water by infected hosts including human and animals, which is to be reported as a largest world's outbreak caused by water-born protozoan parasites [15, 16, 17].

One of the most common sporozoan parasite among cancer patients who undergoing chemotherapy and human immune deficiency virus (HIV) patients is Cryptosporidium [18]. Several epidemiologists have been suggested an association between cryptosporidiosis and cancer in a meta-analysis study, they demonstrate that cryptosporidiosis has a link with malignancy and immune system [19].

Cryptosporidiosis is a type of life-threatening diarrhea in pediatric oncology and other immune-compromised patients. Therefore, effective therapies and strategical control and prevention plans for cryptosporidium infection are an urgent requirement and especially among those patients that suffering from efficient immunity [20].

Another study in Lebanon indicated that the prevalence of cryptosporidiosis was higher among children oncology patients who have diarrhea (14.4%), compared to non-oncology children patients with diarrhea (5.1%). The scientists demonstrated that cryptosporidiosis has close relation with colon cancer [21]. Although, cryptosporidium infection has been attracted attention to large epidemics in developing areas; however, it may be ignored in many regions [22]. Moreover, Cryptosporidium infection has been implicated in diarrheal disease among children and immune-compromised patients.

In a recent study by (Enteric Multi-Centre Study) reported that children in low-income countries are most at risk of life treating acute diarrhea by cryptosporidiosis [23]. The children younger than 5 years are most at risk for Cryptosporidiosis and intestinal disturbance in children < 2 years old [24, 25, 26]. Therefore, cryptosporidiosis considers as the major diarrhea cause and children's mortality after rotavirus infection [27].

Many studies have shown that cryptosporidiosis is a cosmopolitan disease and the occurrence of this parasite is varying around the world. For instance, in America, Australia, and Africa, *Cryptosporidium hominis* is more prevalent, but *Cryptosporidium parvum* has been distributed in the United Kingdom [28]. In poor and underdeveloped countries such as Venezuela, Brazil, Thailand, South Africa, Indonesia, India, Ghana, and Bangladesh, the prevalence of Cryptosporidiosis among children varies from 3% to 13%. In contrast, in developed countries; for example, UK, USA, Canada, Australia, and Denmark, it accounts for only (1% – 4%) of childhood diarrhea [29]. In a large number of European countries, the outbreak of water-born cryptosporidiosis has been recorded at the last two decades, these outbreaks have directly associated with drinking water and contaminated food also swimming pools and animal contact have an important role of cryptosporidiosis outbreak [30,31, 32].

The recent outbreak of intestinal cryptosporidiosis recorded in Finland in 2012 that was such as that in the United Kingdom, it will be directly related to the consumption of fresh salad [33]. While, In the same year, several European countries including (Germany, UK, Belgium, Sweden, Spain, Finland, and the Netherlands) were recorded an abnormal rise in the prevalence of cryptosporidiosis among children and other populations [34].

Many reports indicate that cryptosporidiosis is a community health problem in the Middle East. In Iranian population, a study indicated the role of cryptosporidium in producing diarrhea and mortality among children and immunocompromised individuals, the investigation was designed to identify cryptosporidiosis prevalence, using PCR technique to identify C*ryptosporidium species* genotype and potential risk factors in Isfahan province [35]. There are several articles were published in Iraq about Cryptosporidium. First report of cryptosporidiosis among Iraqi children published by Mahdi et al. in 1996 about Cryptosporidium in Basra [36].

Methods and Materials

A cross-sectional study of Cryptosporidium infection between cancer patients was performed. The data were collected from the patients who visited Hiwa Hospital for cancer patients in Sulaimani Province from April 2019 to October 2019. The stool samples were daily collected and prepared for medical laboratory diagnosis. A total of 300 feces samples from cancer patients and 65 samples from non-cancer patients were collected. The general stool Examination including the direct wet mount (saline wet mount and iodine wet) and indirect permanent staining modified Acid Fast Stain mount (AFS), which is also known as Modified Ziehl Neelsen stain (MZN) were followed for all samples, this stain is a complex stain composed of Carbol Fuchsin, Methylene Blue and Acidic Alcohol.

Also, the immunochromatographic assay was used for Cryptosporidium parasite antigen detection as confirmatory tests in the human stool specimens, the immunochromatographic assay designed for rapid diagnosis of Cryptosporidium parasite in the stool specimens (Rapid-crypto test) with high specificity and sensitivity. The kit consists of a buffer solution for sample dilution and crypto- strip, the instruction of the company was followed (Coris BioConcept, Belgium).

The collected data were statistically analyzed by commercially available software package SPSS version 22 and Graph pad prism, the one way ANOVA and *chi*-square test were used.

Results

Result of the modified Ziehl-Neelsen method (Modified Acid Fast Stain) shows a higher prevalence rate among cancer patients than non-cancer individuals (control group). The infection rate was 17% and 3% among cancer patients and control respectively, this variation of infection was statistically significant ($p<0.05$), the Oocyst received the Carbol Fuchsin stain and it appeared as rose-like with pink color under oil immersion lens (Figure 1).

The immunochromatographic test was used to confirm the positive and negative cases and the sensitivity rate of this test was 86% in comparison with the microscopical examination test (Ziehl Neelsen test) which is 100%.

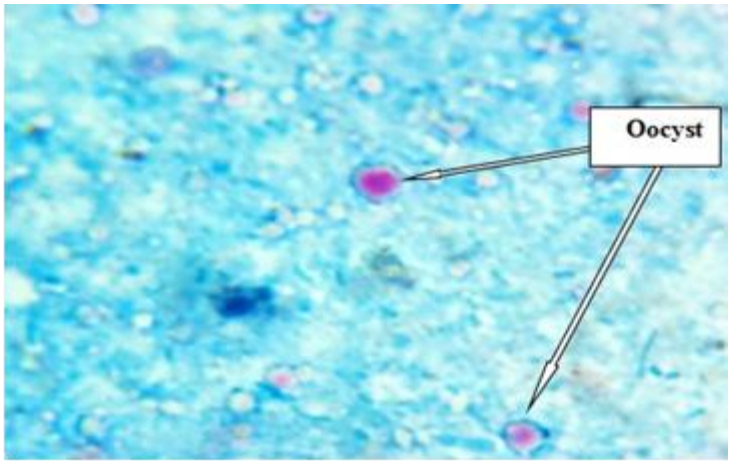

Figure 1: Oocyst of *Cryptosporidium spp.* in thin smear feces of human stained with modified Ziehl Neelsen (100X).

Table (1) shows different types of cancer and cryptosporidiosis, the prevalence rate was higher among blood cancer (6.3%) and Gastric cancer (2.1%) than the other type of cancer while there is no case positive among breast cancer patients and only one case (0.3%) of cryptosporidiosis was detected among gonads cancer patients.

Table (1): Prevalence of cryptosporidiosis to cancer types

Type of Cancer	**Examined Stool Sample**		**Cryptosporidium Positive**		**Cryptosporidium Negative**	
	No.	**%**	**No.**	**%**	**No.**	**%**
Blood cancer	118	39%	19	6.3%	99	33%
Kidney cancer	11	4%	5	1.6%	6	2%
Skin cancer	17	6%	2	0.6%	15	5%
Colon cancer	31	10%	3	1%	28	9%
Breast cancer	33	11%	0	0%	33	11%
Lung cancer	12	4%	4	1.3%	8	2%
Brain cancer	23	8%	3	1%	20	7%
Cervix cancer	11	4%	4	1.3%	7	2%
Gonads cancer	14	5%	1	0.3%	13	4%
Liver cancer	9	3%	3	1%	12	4%
Gastric cancer	21	7%	7	2.3%	14	4%
Total	300	100%	51	17%	249	83%

According to the gender, the prevalence of cryptosporidiosis was 53.3% and 46.7% among males and females respectively, the higher prevalence rate of cryptosporidiosis was found among males than those of females but statistically was no significant (Table 2).

Table 2: The relation between the prevalence of cryptosporidiosis and patients' gender.

Gender	Examined Samples		Positive		Negative		Statistical Analysis
	No.	%	No.	%	No.	%	
Male	160	53.3%	30	10 %	130	43.3 %	$\chi2 = 0.74$ $p = 0.38$
Female	140	46.7%	21	7 %	119	39.7 %	
Total	300	100%	51	17 %	249	83 %	

Table (3) shows the prevalence of Cryptosporidium spp. according to the patient's age. The age of participants ranged from 1 to 88 years and the mean age was 38.76 years. Depending on the age the participants were divided into five age groups (≤10, 11-20, 21-30, 31-40, 41-50 and≥51), the most affected age-groups by the Cryptosporidium spp. were the elders (≥51) person and children (≤10), the prevalence rate was 7% and 3.7% respectively. Statistically, no significant variance was showed among age groups ($P > 0.05$).

Table (3): The prevalence rate of *Cryptosporidium spp*. among cancer patients, according to the age groups

Age (years)	Examined Samples		Positive Sample		Statistical Analysis
	No.	%	No.	%	
≤10	66	22%	11	3.6 %	$\chi2 = 2.47$ $p = 0.78$
11-20	38	12.6%	5	1.7 %	
21-30	18	6%	2	0.7 %	
31-40	25	8.3%	3	1 %	
41-50	39	13%	9	3 %	
≥51	114	38%	21	7 %	
Total	300	100%	51	17%	

Discussion

Cryptosporidium is a protozoan intestinal parasite belong to the sporozoites, cryptosporidiosis are a great factor of waterborne outbreaks and diarrhea among children and immunocompromised patients [37]. The cryptosporidiosis is considered as a common cause of human diarrhea and particularly among the patients who have the immune defect, who received immunosuppressant drugs, cancer patients and children [38]. The Oocyst of Cryptosporidium spp. will be transmitted through the contaminated food, water, and vegetables with feces of infected humans or animals, this

method of transmission also called fecal-oral route [39]. Currently, the modified Ziehl-Neelsen technique for laboratory diagnosis of Cryptosporidium species widely used which has a high specificity 100% and sensitivity 94% [40, 41]. While, the immunochromatographic assay is easy, rapid and simple in comparison to other techniques used for the detection of Cryptosporidium spp. in stool specimens with sensitivity 98.8% and specificity 100% [42, 43]. Modified Ziehl-Neelsen technique is recommended method to oocyst detection, but it needs the experience and time-consuming. In the current study, the less sensitivity of the crypto-strip method 86% compares to the modified Ziehl- Neelsen method, this variation in the sensitivity rate of the immunochromatographic test which is designed for cryptosporidium antigen detection in human feces, maybe due to the sample storage and sample handling process also the immunochromatographic test quality may be varied depending on the supplier company and manufacture company.

The rate of *Cryptosporidium spp.* prevalence for participated patients in the current study was 17%, while the prevalence rate was 3% among non-cancer patients (control group), in contrast to our results Aksoy and his colleagues in Turkey [44] and Tappeh et al. in Iran [45] reported low prevalence rate (4%) and (4.2%) respectively among cancer patients, this low rate of infection may be due to environmental factors and sample size which was less than the current investigation. High prevalence of Cryptosporidiosis in the current study may be due to lifestyle, health status, immunological condition, outdoor and indoor pollution of environment, personal hygiene habits, individual and community health education about communicable diseases, management of sewage water, drinking water, and economic status of poor families.

In Europe, there is a low prevalence rate of *Cryptosporidium spp.* Pettoello-Mantovani et al. in Italy, revealed a low rate of cryptosporidiosis prevalence (4.7%) [46], the low prevalence rate in Italy is multifactorial, Italy is considered as a developed country which reached adequate sanitation, clean water, suitable sewage treatment, high economic status, high level of health education.

The high prevalence rate of Cryptosporidium among cancer patients was reported in each of Egypt (24%) by Hassanein et al. [47], in Saudi Arabic (70%) reported by Sanad et al. [48] and in Pakistan (80%) reported by Baqai et al. [49]. The high prevalence rate of cryptosporidiosis in these countries may be returned to diagnosis techniques and low quality of the diagnosis kits or equipment, lifestyle, culture, lack of sanitation facilities and small sample size. On the other hand, in the current study, although males patients showed slightly higher prevalence rate (10%) in comparison to females patients (7%), this varies according to the sex, statistically was not significant for cryptosporidiosis among cancer patients.

Our finding is supported by Sulżyc-Bielicki et al.[17] in Poland, Mohaghegh et al. [50] in Iran and Baqai et al. [49] in Pakistan. They stated that the male most prone to parasite exposure compare to females due to the nature of men's lifestyle such as type of occupation and work environment. While the results of Sherchand and Shrestha [47] in Nepal were strongly disagreed with the current study they reported a high prevalence rate in females than in males for all age groups, they explained this high rate of cryptosporidiosis among females due to close contact of the female with children than males, and females often spend more time with child care [47].

Regarding the age groups of cancer patients with cryptosporidiosis infection, the current study recorded high positive results for cryptosporidiosis among the patients who are more than 50 years old (≥ 51), out of 51 positive samples, 21 positive samples recorded with age group older than 50 years old (≥ 51) with the prevalence of 7%, which is higher than the other age groups, This finding disagrees with Mohaghegh et al. [51] they reported high prevalence rate among young ages the reason may return to that in the present study the cancer patients were included and usually cancer disease more common in old age, while in Mohaghegh et al. Study hemodialysis patients included which affect all ages in the community.

In the current study, the highest infection rate of 37.2% was recorded among patients who had blood cancer in comparison to the other types of cancer, it may be due to the type of chemotherapy. The patients with the esophagus, stomach, intestinal, rectum cancer may be received chemotherapy directly through the digestive system, then all microorganism pathogens and normal biota of the intestine will be affected directly more than those they received the chemotherapy through blood or other modes of patient treatment.

Conclusion

In the conclusion, cancer patients are most at risk for cryptosporidiosis and they are suffer from cryptosporidiosis than the healthy individual in Sulaimani Province, the Kurdistan Region of Iraq is an endemic of cryptosporidiosis, because of accuracy lack of laboratory diagnosis of stool specimen in the region, a further molecular investigation is required for species and molecular characterization of *Cryptosporidium*.

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Ethical Clearance: All experimental protocols were approved under the Department of Community Health, Technical College of Health, Sulaimani Polytechnic University and all experiments were carried out in accordance with approved guidelines.

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