

Detection of *Cryptosporidium parvum* by modified acid-fast stain among cancer patients in Thi-Qar province

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Abstract—: *Cryptosporidium parvum* is a widespread opportunistic parasite belonging to the family Apicomplexa. It completes its life cycle in a single host and is frequently found in immunocompromised individuals, especially in patients with cancer. The objectives of this research were to determine the prevalence of *Cryptosporidium parvum* infection among cancer patients in the Thi-Qar Province of Iraq, using the modified Ziehl-Neelsen (ZN) stain. The study included 300 stool samples, with 200 collected from patients with cancer and 100 from healthy individuals (the control group). Based on the modified acid-fast stain, the results showed that the rate of infection with *Cryptosporidium parvum* among cancer patients was 54.0% (108/200), compared to 30.0% (30/100) in the control group. There were statistically significant differences between the two groups. The percentage of infection among male cancer patients was higher than females, but there were no significant differences between them, with percentages of 57.14% and 52.03%, respectively. Regarding age groups, *Cryptosporidium parvum* affected individuals across all age groups in different proportions. The study showed that the highest infection rate among the age groups was observed in individuals aged ≥60 years, at a rate of 64.44%. The lowest infection rate was found in the age group ≤20 years, at a rate of 35.71%. Analyzing the number of chemotherapy doses received, the results showed that the infection rate was higher in patients who received half or more than half of the chemotherapy doses. Among those who received 16-20 doses, the infection rate was 64.0%, and for those who received 21-25 doses, it was 62.50%. The lowest rate was observed in patients who received 1-5 doses, with a percentage of 49.02%.

Keywords— Cryptosporidiosis, acid-fast stain, Thi-Qar province, *cryptosporidium parvum*.

I. INTRODUCTION

Cryptosporidium parvum is the most widespread opportunistic intestinal parasite worldwide causing diarrhea as well as a common cause of death and a significant contributor to a number of intestinal disorders. [1].

Cryptosporidium parvum causes Cryptosporidiosis, one of the most common intestinal diseases. Cryptosporidiosis is a widespread opportunistic disease affecting both humans and animals, and is the fourth leading cause of diarrhea worldwide especially in people, who are immunocompromised [2], [3].

It is one of the four leading diseases to acute diarrhea and death in infants and young children [4], and is a prominent cause of diarrhea worldwide in both humans and animals [3], and the second leading cause of diarrhea-related death in children under five [5], [6]. While immunocompetent individuals find it unpathological or asymptomatic, which prevents them from recognizing that they have the disease [7].

There are two main ways of *Cryptosporidium parvum* transmission. The first is indirectly by eating food contaminated with the parasite or swallowing feces in water and the second is directly after contact with people and animals that carry the parasite [3]. *Cryptosporidium parvum* is a single-celled parasite and member of the Apicomplexa family with a complex biological life cycle consisting of asexual and sexual reproduction that is within a single host and does not require an intermediate host [8], the size of this parasite is 4-5 micrometers, in addition. It is smaller than the size of a red blood cell [9]. Among the distinguishing characteristics of Oocysts is that they can withstand harsh



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climatic conditions for six months and temperatures of up to 10 for twelve weeks, and stay in moist soil for eight weeks, as well as They can tolerate chlorine and are resistant [10]. The aim of this study is detection of *Cryptosporidium parvum* microscopically by acid-fast stain in cancer patients in Thi-Qar province and studies some of the factors affecting its spread, such as age, gender, and others.

II. MATERIALS AND METHODS

A. Collection of samples

The current study was conducted on (200) stool samples from cancer patients who received chemotherapy who attended the Thi-Qar Oncology Center in Al-Haboubi Teaching Hospital and Al-Nasiriyah Teaching Hospital, in addition to (100) samples from healthy people as control group for period between October 2022 to February 2023, for detection of *Cryptosporidium parvum*. After obtaining the consent of all patients, an information sheet was prepared and designed according to a questionnaire covering various information such as gender, age, number of chemotherapy doses, and type of cancer. Stool samples were collected using sterile containers, and all required patient information was written on them.

B. Laboratory examination

The parasite was diagnosed by: direct microscopy and modified acid rapid staining.

- **Direct wet mount technique**

A slide was taken and a drop of Normal saline solution was added to it, than 1 mg of stool was taken with a stick and mixed well, after that a sliding cover was placed on it and the examination was performed directly at 10x or 40x magnification [11].

- **Modified Acid-Fast staining technique**

The method was done according [12] as following:

Appropriate amount of stool was taken to make a swab by mixing it with a few drops of water using a stick and sticking it on the slide and immersing it in carbolfochin dye for five minutes than heating the slide for a few seconds until the carbolfochin evaporates .The slide was washed with water to remove the rest of the dye .The slide was immersed in rubbing alcohol for one to three minutes .The slide was washed again with water to remove the alcohol, and finally immersed in methylene blue for one minute, and the slide was washed with water to remove methylene blue. The slide was dried by air. Examine the slide with a 40X and then 100X lens to identify parasitic Oocysts that appear dark pink or purple. And when using the 100X lens, special oil must be used for it.

C. Statistical analysis

The data of the present study were statistically analysis by using SPSS (Statistical Package of Social Science version 26) based in using both descriptive and non-parametric Chi-Square at p. value < 0.05.

III. RESULTS

- **Diagnosis of *Cryptosporidium parvum*:**
- **Microscopy results by direct wet mount technique:**

All samples were examined by direct smear, and all results were negative.

- **Microscopy results using modified acid fast stain.**

The results of the microscopic examination of positive stool samples, which contain Oocysts , after staining with acid fast stain , showed that the Oocyst are spherical in shape, reddish-purple in color, while the rest of the stool appears blue, as shown in the figure (1):

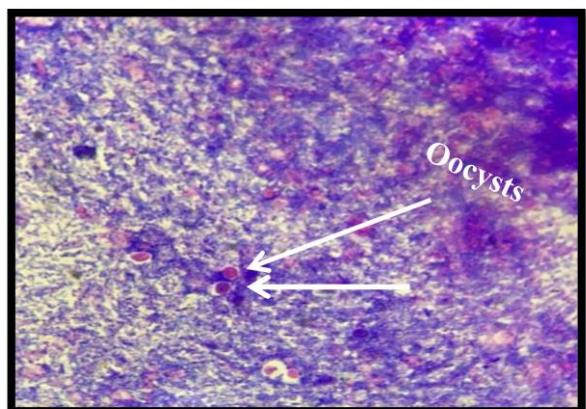


Figure (1): Oocysts of *Cryptosporidium parvum* (white arrow) isolated from human feces staining with acid fast stain (100X)

- **Percentage of infection with *Cryptosporidium parvum***

According to the modified acid stain, cancer patients had a higher prevalence of *Cryptosporidium parvum* compared to the control group, and the infection rate was 54% and 30%, respectively. The statistical analysis showed significant differences between them ($p<0.05$) as shown in table (1):

Table (1): Percentage of infection with *Cryptosporidium parvum* in cancer patients and control group:

Group	Positive		Negative		Total	
	No.	%	No.	%	No.	%
Cancer Patient	108	54.0	92	46.0	200	66.67
Control	30	30.0	70	70.0	100	33.33
Total	138	46	162	54	300	100
Cal χ^2 = 11.8	Tab χ^2 = 3.84		DF= 1	p. value 0.001		

The results of the current study indicated that the percentage of infection with *Cryptosporidium parvum* among males was higher than that of females, and the percentage of infection was 57.14% (44/77) and 52.03% (64/123) respectively. The statistical analysis showed non-significant differences between them ($p<0.05$) as shown in table (2):

Table (2): Percentage of infection with *Cryptosporidium parvum* among cancer patients according to Gender:

Gender	Positive		Negative		Total	
	No.	%	No.	%	No.	%
Male	44	57.14	33	42.86	77	38.50
Female	64	52.03	59	47.97	123	61.50
Total	108	54.0	92	46.0	200	100
CalX ² = 0.504	TabX ² 3.84	DF= 1	p. value 0.478			

Table (3) shows that the rate of infection with parasite Oocysts depends on the age group, as it was noted that the highest infection rate was in the age group ≥ 61 , followed by the age group 31-40, and the lowest infection rate in the age group ≤ 20 . The infection rates among them, respectively, were as follows: 64.44%, 56.66%, and 35.71%. The statistical analysis showed significant differences between them ($p<0.05$).

Table (3): The percentage of *Cryptosporidium parvum* infection among cancer patients based on age:

Age group	Positive		Negative		Total	
	No.	%	No.	%	No.	%
≤ 20	5	35.71	9	64.29	14	7.0
21-30	11	47.83	12	52.17	23	11.50
31-40	17	56.67	13	43.33	30	15.0
41-50	20	48.78	21	51.22	41	20.50
51-60	26	55.32	21	44.68	47	23.50
≥ 61	29	64.44	16	35.56	45	22.50
Total	108	54.0	92	46.0	200	100
CalX ² = 18.3	TabX ² = 11.07	DF= 5	p. value 0.003			

The results of the current study indicate that the infection rate of *Cryptosporidium parvum* is highest among people who received 16-20 doses of chemotherapy, followed by people who received 21-25 doses, and the lowest infection rate was among people who received 1-5, and the rates were as follows: 64.0 %, 62.50%, and 49.02%. Respectively .The statistical analysis showed non- significant differences between them ($p<0.05$) as shown in table (4):

Table (4): Percentage of infection with *Cryptosporidium parvum* among cancer patients according to the number of doses of chemotherapy:

Number of Dose	Positive		Negative		Total	
	No.	%	No.	%	No.	%
1 - 5	25	49.02	26	50.98	51	25.50
6 - 10	28	50.91	27	49.09	55	27.50
11 - 15	20	57.14	15	42.86	35	17.50
16 - 20	16	64.0	9	36.0	25	12.50
21 - 25	10	62.50	6	37.50	16	8.0
26 - 30	9	50.0	9	50.0	18	9.0
Total	108	54.0	92	46.0	200	100
CalX ² = 8.70	TabX ² = 1107	DF= 5	p. value 0.121			

The results indicated that the incidence of *Cryptosporidium parvum* was highest among patients with breast cancer, followed by those with prostate cancer, and the lowest incidence among patients with cervical cancer. The rates were as follows: 68.63%, 60.0%, and 35.29%, respectively. The statistical analysis showed significant differences between them ($p<0.05$) as shown in table (5):

Table (5): The percentage of *Cryptosporidium parvum* infection among cancer patients based on the type of cancer:

Type of cancer	Positive		Negative		Total	
	No.	%	No.	%	No.	%
Breast Cancer	35	68.63	16	31.37	51	25.50
Respiratory Cancer	4	44.44	5	55.56	9	4.50
Lymphoma	8	47.06	9	52.94	17	8.50
Gastrointestinal Cancer	12	50.0	12	50.0	24	12.0
Bone Cancer	5	50.0	5	50.0	10	5.00
Brain Cancer	4	44.44	5	55.56	9	4.50
Cervical Cancer	6	35.29	11	64.71	17	8.50
Blood Cancer	22	56.41	17	43.59	39	19.50
Thyroid Cancer	3	50.0	3	50.0	6	3.0
Prostate Cancer	3	60.0	2	40.0	5	2.50
Bladder Cancer	3	50.0	3	50.0	6	3.0
Pancreas Cancer	3	42.86	4	57.14	7	3.50
Total	108	54.0	92	46.0	200	100
CalX ² = 34.0	TabX ² = 19.68	DF= 11	p. value < 0.001			

IV. DISCUSSION

Many intestinal parasites infect more than 3 billion people worldwide, causing 450 million people to become ill. *Cryptosporidium* spp. is an opportunistic parasite and one of the main causes of diarrhea, especially in immunocompromised people such as those with cancer [13]. This study included examining 300 stool samples, 200 samples from cancer patients who received chemotherapy, and 100 samples from healthy persons as a control group. Microscopic method was used for the diagnosis, includes a direct smear and a modified acid-fast stain. The results were negative for all the examined samples by using the direct smear, which means *Cryptosporidium parvum* was not detected in this way due to the small size of the Oocysts and requires a specific method to detect them. Based on modified the acid-fast stain, which is characterized by simplicity, low cost, and the ability to clearly show the internal details of the Oocysts, but it, takes a long time and requires experience. It showed that the Oocysts are spherical in shape, with a reddish-purple color, while the rest of the stool appears in blue. The results based on the modified

Acid-fast stain showed that the incidence of *Cryptosporidium parvum* among cancer patients was

54.0% (108/200) compared to the control group, which was 30.0% (30/100), and there were statistically significant

Differences between them. The results of this study are consistent with [14] from Sulaymaniyah Province in Iraq. He showed that the prevalence of Cryptosporidiosis in cancer patients and the control group is 17% and 3%, respectively. This study also agreed with [15] from Iran, his study showed that the rate of Cryptosporidiosis in cancer patients undergoing chemotherapy was higher than the control group by 3.8% and 1.5%, respectively. In contrast to [16] from Australia, his study did not find any infection with *Cryptosporidium parvum* in cancer patients compared to the control group, where the infection rate was 13%, and similarly [17] from Mexico indicated that the percentage of infection with *Cryptosporidium parvum* in people with cancer Less than the control group, where the percentage was 12.9%, 26%, respectively. The rise in the current study may be due to lifestyle, health status, immune status, type of treatment used, environmental pollution inside or outside the home, personal hygiene of the people, and health culture around Infectious diseases, economics, and management of drinking water and Sanitation, until cryptosporidiosis becomes severe and chronic, unlike the patients in the control group, whose immunity is high, and the infection is self-limiting [14]. The infection rate among males was higher than among females, but there were no statistically significant differences between them, as the percentage was 57.14% and 52.03%, respectively. This study is consistent with [14] from the Sulaymaniyah province in Iraq, where he indicated that the infection rate in males was higher than in females, and the infection rate was (10%) for males and (7%) for females. It also agreed with [18] from Babil province in Iraq, where he indicated that the incidence. In males is 6.2% and 1.1%, in females as well as [19] from Palestine, where he indicated in his study that the infection rate was 11.3% in males and 9.7% in females. On the other hand, this study differed with [20] from Al-Qadisiyah province in Iraq, where he mentioned that the infection rate in females was higher than that of males, 56.6% and 43.3%, respectively. As well as [21] from the city of Isfahan in Iran, he stated that the infection rate in females was 2.6% and in males 2.0%. The high incidence among males in this study may be due to several factors, including the man's lifestyle, and their continuous contact with the external environment, which plays an important role. In the transfer of Oocysts, the type of feeding, eating exposed foods in public places, lack of interest in personal hygiene, etc. [22]. On the other hand, the results confirmed that *Cryptosporidium parvum* it affects all age groups in different proportions. The study showed that the highest infection rate among the age groups reached ≥ 60 years at a rate of 64.44%, and the lowest infection rate among the age group ≤ 20 years at a rate of 35.71%. This study agrees with [23], from Iran, who indicated in his study that the highest infection rate was in the age group of 40-60 years, and [24], from the city of Bushehr in Iran, where he indicated that the age group is 51-70 year-olds recorded the highest rates of *Cryptosporidium parvum* infection, as

well as [14], from the Sulaymaniyah Province in Iraq, he recorded an increase in infection rates among patients over the age of ≥ 51 years, with a prevalence rate of 7%, which is higher than other age groups. On the other hand, this result is not consistent with [25], from Turkey, , he indicated that the infection abounds in infected people between the ages of 1-15 years, at a rate of 28.7%, and [26] from Nigeria indicated that the prevalence rate was higher in the age groups from 1-5, with a rate of 37.3%, [27] from Iran, where he indicated that the highest infection rate was in people under the age of 20. The reason for the high incidence in age group ≥ 60 may be due to a decrease in people's immunity due to their old age, the type of treatment used, or the environment they are in, and the lack of adequate awareness and culture [14]. As for the number of doses of chemotherapy the results showed that the infection rate of *Cryptosporidium parvum* It was higher in patients who received half and more than half of the doses of chemotherapy, where the percentage of those who received 16-20 doses (64.0%) and the percentage of those who received 21-25 doses (62.50%) and the lowest rate was in patients who received 1-5 doses Their percentage was (49.02). This result is consistent with [28] from Iran showed an increase in parasites in cancer patients who completed half of the chemotherapy cycles by 22.2%, [29] from Malaysia who showed an elevation of parasites in cancer patients during chemotherapy doses using the same classification. One of the adverse effects of chemotherapy is the effect on the immune system and immune function, which increases the risk of parasitic infections [30], [31] and the reaction of the patient's body to potentially harmful agents. The side effects of chemotherapy drugs may strengthen his immunity and help them successfully fight off parasitic infections in nature [29]. As for the type of cancer, the results showed of *Cryptosporidium parvum* was higher among women with breast cancer, at 68.63%, followed by those with prostate cancer at a rate of 60.0%. And the lowest among women with cervical cancer, 35.29%. This study is consistent with [32] from Mosul Provence in Iraq, where he indicated that the highest rate of parasite infection was among patients with breast cancer, at a rate of 35.85%. On the other hand, this study is inconsistent with [14] from Sulaymaniyah Provence in Iraq, where he indicated that the highest rate of parasite infection was among patients with leukemia by 37.2%, [33] from Saudi Arabia, where he indicated that the highest infection rate was among patients with colon cancer with a rate of 100%. The reason for the high rate of infection with parasites in patients in patients with breast cancer due to several reasons including the type of chemotherapy used, low immunity because women have less immunity than men or because of the constant presence with children, or the quality of nutrition or the effort involved in household chores.

V. CONCLUSION

Cryptosporidium parvum is one of the causes of diarrhea among cancer patients in Thi-Qar Province It is higher than its prevalence among healthy people, and the microscopic

examination using a modified acid-fast dye is better than the direct smear for the diagnosis of *Cryptosporidium parvum*.

CONFLICT OF INTEREST

Authors declare that they have no conflict of interest.

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