

Identification of *cryptosporidiumparvum* in colorectal cancer tissue in Thi-Qar province



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Abstract— Colon cancer is one of the most common cancers around the world. It is classified as the third cancer that affects humans. Many studies show risk factors for cancer. This study was designed as a reference study (retrospective). This study was conducted in Dhi-Qar Governorate at Al-Hussein Hospital, peace be upon him, and included the collection of colon and rectal tissue samples immersed in paraffin wax for patients with colon cancer, during the period from October 2020 to April 2021 at the age of 20 years. The aim of the study is to diagnose the presence of the cryptosporidium parasite in the tissues of colorectal cancer patients by using polymerase chain reaction (PCR). The results of the polymerase chain reaction showed infection in *Cryptosporidium* parasite 13.3% (13) positive samples out of 75 samples, and infection in *Cryptosporidium parvum* 9.2% (9) positive samples. The study also recorded a discrepancy between infection rate and gender. The infection rate of *Cryptosporidium* (8) samples 66.7% and *Cryptosporidium parvum* (5). The study also showed the most prevalent stages of cancer and the most common stages in which *Cryptosporidium* are present. The study recorded the second stage of colon cancer patients with the most diagnosed stages, as well as the presence of *Cryptosporidium* in it (4) 33.3%. But *Cryptosporidium* recorded the most infection in the first stage of colon cancer (4) 50%.

Key words: *Cryptosporidiumparvum* , molecular, diagnosis, detection.

Introduction

Cancer is a group of diseases marked by uncontrolled cell growth. There are over 100 different types of cancer, each of which is named based on the type of cell that is infected first. When compromised cells divide uncontrollably, they form tumors, which are lumps or masses of tissue (Communications, 2014) (Tyagi *et al.*, 2017). Colon cancer (CRC) is just the third most common carcinoma, preceding lung and breast cancers, about 1.4 million new cases recorded in 2012. (World Cancer Research Fund International) (Arafa and Farhat, 2015). Bowel cancer starts when healthy cells in the colon or rectum floor migrate and expand uncontrollably, leading to the establishment of a tumor. Tumors can be malignant or non - cancerous. A cancerous lump has the capacity to form and invade nearby tissues. (NHS Cancer Screening Programmes, 2012). *Cryptosporidium* infection has numerous unknown pathophysiological mechanisms. Recent study has revealed that *Cryptosporidium parvum* infection promotes cytoskeleton remodeling and actin reorganization via the participation of several intracellular signals such as PI3K, Cdc42, and GTPase. Infection with *C. parvum* stimulates NF- κ B, which is thought to enhance anti-apoptotic pathways as well as transmit oncogenic signals to epithelial cells (Sawant *et al.*, 2020). Cryptosporidiosis is just a parasitic disease caused by the pathogen *Cryptosporidium*, and it impacts several vertebrate species, such as humans, causing acute gastroenteritis, stomach discomfort, and diarrhea (Gerace *et al.*, 2019). The much more common infection is in the gastrointestinal system, with diarrhea being the most common symptom. Several hosts, particularly birds, are prone to respiratory illness. Other locations, most commonly in birds and immune compromised humans, can be impacted as well (ADL S.M, SIMPSON A.G, LANE C.E, 2018). Despite the fact that the number of internationally documented cases of cryptosporidiosis has increased in recent years to 3 instances per 100,000 individuals, many indicators (such as medical

symptoms) indicate that the disease's prevalence is 100 times more than the number of cases discovered (Gerace *et al.*, 2019). According to feces analysis, *Cryptosporidium* is identified in 1-4 percent of gastroenteritis patients in Europe and North America, and 3 to 20 percent in Africa, Asia, Australia, South, and Central America. The prevalence rises in developing nations in late summer and early spring. In industrialized nations, children under the age of five and young adults have the highest frequency (Yuan *et al.*, 2016). Diagnosis of the *cryptosporidium* in the same tissue and proving the relationship between the parasite and the prognosis of *colon cancer* as well as the comparison between the PCR method and the tissues staining by Ziehl-Nelson stain for diagnosing cryptosporidium.

Materials and methods

Study design and sample collection

A retrospective study was performed in histological unit in the laboratory of Al -Hussein Teaching Hospital / Thi-Qar-South of Iraq, and Cancer Researches Unite/ College of medicine–University of Thi-Qar during period between (October 2020 to April 2021).

A total of 98 formalin fixed paraffin-embedded tissues were collected included 75 colorectal cancer tissue and 23 benign colon tumors as diagnosed by specialist, all samples were taken from patients attending to Al-Hussein teaching hospital and Ibn Al-Bitar laboratory with ages ranging from 20 to 80 years. Demographic data including: age, address, gender were recorded for each patient under study. Samples were first used to extract DNA for PCR technique and to detect the parasite of interest by Ziehl-Nelson stain.

DNA extraction and sequences primer

Genomic DNA was extracted from FFPE tissue samples by using GSYNCTM DNA Extraction Kit. The primers were specific for amplification of genes of interest that detect (*Cryptosporidium* genus, *Cryptosporidium parvum*).

18S rRNA, *Cryptosporidium* (5'-AAC CTG GTT GAT CCT GCC AG-3'-5'-TGA TCC TTC TGC AGG TTC ACC TA-3').

BCOWP, *C.parvum*(5-ACCGCTTCTCAACAACCATCTTGTCCTC-3- 5-CGACCTGTTCCCACTCAATGTAAACCC-3).

All PCRs and sequencing reactions were performed on a PCR System 9700 (Applied Biosystems).

Statistical analysis

The following tests were used to analyze the data: The statistical investigations of results were done by the help of Minitab using version Spss statistical bundle. Chi-Square test was utilized to discover the P-value between different markers with other variables. Correlation coefficient test was utilized to discover the correlation among different markers with other parameters. The level of significance was 0.05 (or less) in all statistical testing, (p value less than 0.05).

Results

Distribution of *Cryptosporidium Parvum* infection according to age groups by using PCR method:

Our study showed that there were the high *Cryptosporidium Parvum* infection percentage in (61- 70 years) groups in Cancer tumor (37.5%) and Benign tumor (33.4%), While the lowest infection was in the (31- 40 years) age group in Cancer Tumor (0.0%) and Benign Tumor (11.1%). The result also revealed of a moderate positive relationship between age and *Cryptosporidium Parvum* infection, And the results statistically significant difference in *Cryptosporidium Parvum* infection with age as table (1).

Table 1: Distribution of *Cryptosporidium Parvum* infection according to age groups.

<i>C. parvum</i> Age Group	Cancer Tumor		Benign Tumor		Total	
	No.	%	No.	%	No.	%
31-40 Y	0	0.0	1	100	1	11.1
41-50 Y	2	25.0	0	0.0	2	22.2
51-60 Y	1	12.5	0	0.0	1	11.1
61-70 Y	3	37.5	0	0.0	3	33.4
Above70 Y	2	25.0	0	0.0	2	22.2
Total	8	100	1	100	9	100

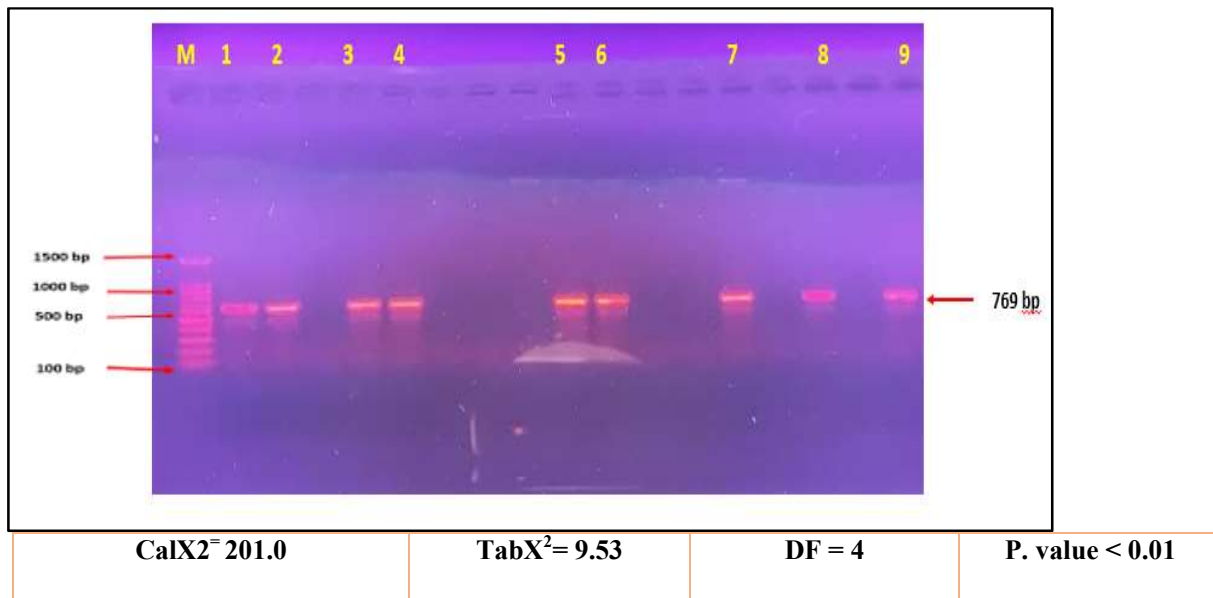


Fig 1: Agarose gel electrophoresis for *Cryptosporidium Parvum*. Image that showed the PCR product of 769 bp. Lane M: DNA analysis of small subunit (18s) ribosomal RNA gene in *Cryptosporidium Parvum* from human tissues samples.

Distribution of *Cryptosporidium* and *Cryptosporidium Parvum* infection according to Gender groups by using PCR method:

Table (2) show distribution of *Cryptosporidium* and *Cryptosporidium Parvum* infection depended on gender by use PCR. The result were the highest infection in male more than female *Cryptosporidium* (66.7%) and *Cryptosporidium Parvum* (62.5%). The result also showed relationship between this parities infection and gender, statistically significant difference infection with male as table (2).

Table (2) Distribution of *Cryptosporidium* and *Cryptosporidium Parvum* infection according to gender groups.

<i>Cryptosporidium</i> Gender	Cancer Tumor		Benign Tumor		Overall-total	
	No.	%	No.	%	No.	%
Male	8	66.7	1	100	9	69.2
Female	4	33.3	0	0.0	4	30.8
Total	12	100	1	100	13	100
CalX ² = 39.251	TabX ² = 3.84		DF = 1		P. value < 0.01	
<i>C. parvum</i>						
Male	5	62.5	1	100	6	66.7
Female	3	37.5	0	0.0	3	33.3
Total	8	100	1	100	9	100
CalX ² = 46.395	TabX ² = 3.84		DF = 1		P. value < 0.01	

Distribution *Cryptosporidium parvum* infection according to stage by using PCR method:

Table (3) show distribution of *Cryptosporidium parvum* infection depended on stage of tumor explain the stage I higher prevalence from other stages of *Cryptosporidium parvum* infection, That recorded (50.0%) in Cancer Tumor, While show the result stage 0 more prevalence(100.0) than other stages in Benign Tumor, As well explain stage IV(0.0%) low stage infection from other in both Cancer and Benign Tumors. The result show statistically significant difference between stage and *Cryptosporidium parvum* infection and It is noted that the infection rate of the parasite is high in the stage I as table (3).

Table (3) Distribution *Cryptosporidium parvum* infection according to stage by using PCR method.

<i>C. parvum</i> Stages	Cancer Tumor		Benign Tumor		Total	
	No.	%	No.	%	No.	%

Stage 0	1	12.5	1	100.0	2	22.2
Stage I	4	50.0	0	0.0	4	44.4
Stage II	1	12.5	0	0.0	1	11.2
Stage III	2	25.0	0	0.0	2	22.2
Stage IV	0	0.0	0	0.0	0	0.0
Total	8	100	1	100	9	100
CalX² = 154.981	TabX² = 9.35		DF = 3		P. value < 0.01	

Discussion:

Our study showed that there were the high *Cryptosporidium Parvum* infection percentage in (61- 70 years) groups in Cancer (37.5%) and Benign tumor (33.4%). The result also revealed of a moderate positive relationship between age and *Cryptosporidium Parvum* infection, And the results a significant difference in *Cryptosporidium Parvum* infection that increase age. The present study agreed with the study Digestive biopsies fixed in formalin and embedded in paraffin were collected in Tripoli, Lebanon, between 2012 and 2013 from groups of patients 72 samples the average age (18-92 years), this study show age group (18-50) higher infection(Osman *et al.*, 2017). Agreed with the study included 108 consecutive patients with colorectal cancer median age of 65 years, this study recorded (61-70 years) age group more infection in parasitic (Sulżyc-Bielicka *et al.*, 2018). Agree with the study included 195 patients average age (50- more than 60) years with diagnosed gastrointestinal cancers of Jilin University during January-July, 2018. The study recorded (>60) age group(13.33%) higher infection in parasite (Zhang *et al.*, 2020).

Because *Cryptosporidium* is an opportunistic agent that causes considerable morbidity and death in immune compromised patients, persons with cancer may be at a higher risk of contracting this parasite, especially if their immunosuppression is severe. The samples used in this investigation came from HIV-negative people who had just been diagnosed The risk of opportunistic infection due to the presence of cancer as an immune debilitating disease appeared to be minimal at the time(Osman *et al.*, 2017).

Patients with malignancies frequently have transitory or permanent immune deficiencies caused by the neoplastic illness, oncological therapy, or the elderly more likely to be infected with *Cryptosporidium parvum*(Sulżyc-Bielicka *et al.*, 2018).Infection with *Cryptosporidium parvum* is a serious danger in relation to human health or the elderly It is one of the most significant causes of It causes diarrhea and can be fatal in immune compromised people(Zhang *et al.*, 2020).

Cryptosporidium and *Cryptosporidium Parvum* infection depended on gender by use PCR. The result were the highest infection in male more than female *Cryptosporidium*(66.7%) and *Cryptosporidium Parvum* (62.5%).

The result also showed relationship between this parities infection and gender, a significant difference infection with male. Our study compatible with the study that (90) Samples were taken from both sexes and of difference ages for the residents of Baghdad governorate/Iraq in private laboratories in 2019. The study showed infection in male higher from female, recorded 50 sample positive in male (44.44%)(Waheeb *et al.*, 2020). Agreed with the study Fifty samples were collected from patients of both genders with different ages attended the four teaching hospitals (Al-Sadder, Al-Hakeem, Al-Zahra and Al-Frat Al-Awsat) in Al-Najaf city /Iraq the study show male infection higher than female was recorded the male infection 16(55.2%) and female 13(44.8%) (Sayal, 2019).

Also similar the study included 108 consecutive patients with colorectal cancer. The group had a median age of 65 years and consisted of 42 women and 66 men. The study show male infection more than female was recorded the male infection 66(61.1%) (Sulżyc-Bielicka *et al.*, 2018). Agree with the study Fifty samples were collected from Jinnah Postgraduate Medical Centre and Kidney Centre Karachi. The study record male infection high (57.10%) from female infection(33.30%)(Baqaietal., 2005).

Disagree with the study collected randomly by the Korea association of health for the fifth national survey on the prevalence of intestinal parasitic infection in Korea collect 751 samples, the study record female more infection from male the rate female infection 133 sample and male 115 sample (Jong-Yil chai, 2011). Also disagree with the study was prospectively conducted in three university hospitals in Isfahan from August 2007 to June 2008. Children aged one month to 10 years presenting with acute or persistent diarrhea collect 606 samples, the study show female infection (57.1%) higher than male infection (42.8%)(Saneianet *al.*, 2010).

The current investigation found that male gender was an independent factor linked with a high incidence of *Cryptosporidium* spp. infections in all participants (patients with colorectal cancer and controls). The likelihood of *Cryptosporidium* spp According to the European register, the male-to-female ratio of *Cryptosporidium* infections is 3:1 Gender differences are thought to be influenced by both hereditary and environmental factors(Sulżyc-Bielickaet *al.*, 2018). *Cryptosporidium* and more instances were in men, according to our findings. Cancer patients are more immune compromised and are more likely to be infected with *Cryptosporidium*, according to our findings(Baqaiet *al.*, 2005).

The results agree with the study the Iraqi Ministry of Health, that were gathered from all governorates. The show stage I more infection(Hussain and Lafta, 2020). Agree with the study included 108 consecutive patients with colorectal cancer. Pomeranian Medical University. The group had a median age of 65 years. The study recorded stage I higher infection(Sulżyc-Bielickaet *al.*, 2018). Agree with the study, analyzing trends in population incidence rates of colorectal cancer in England. The study explain the stage I more infection(Donaldson, 2019).

Disagree with The Behavioral Risk Factor Surveillance System (BRFSS) is an annual, state-based, random-digit-dialed telephone survey of the civilian, no institutionalized adult population aged ≥ 18 years that collects information on health risk behaviors, preventive health practices, and health care access in the United States, the study recorded stage II more infection (Joseph et al., 2020).

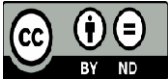
The first stage of colorectal cancer is easier to diagnose since it begins with a big bowel illness and the appearance of apparent symptoms on the patient, as well as the simplicity of detection by microscopy. The rest of the stages are advanced stages of the disease, and they usually appear after the diagnosis of the disease, that is, they are advanced stages of infection(Wong et al., 2019).

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