

# Study of Some Immunological Markers of People with Parasitic Infestation and their Relationship to Colorectal Cancer

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## Abstract

The aims of this study to find the link between each of the parasites (*E. histolytic* and Giardiasis) infection and intestinal cancer, This study contain 4 groups :- Group (G1) People with cancer and diarrhea due to the parasite (42) Group (G2) People with cancer and diarrhea without parasites (8) Group (G3) People with diarrhea and parasites (24) and Group (G4) Control (30), blood samples were collected from patients suffering from diarrhea and parasite infection of both types, as well as cancer, Elisa kits were used in our study the proteins (mucin 2, TFF3, CALP) level were measure by using Elisa kit that provided from the China Origin Company Elabscience. The results of the current immunological study showed the level of serum (mucin 2, TFF3, CALP) among the study groups, where the levels of mucin 2 values were (14305.00), (10595.00), (13145.00) and (9350.00) in group (1), and group (2), group (3) and group (4) respectively, and the level of serum values (TFF3) (43.15), (11.90), (29.65) and (8.60) with the same sequence of groups above, and the level of serum values CALP (16320.00), (11220.00), (15275.00) and (8690.00) with the same sequence of groups above as well. The results of the statistical analysis showed that there is a significant difference between the study groups below the probability level ( $P < 0.001$ ). (mucin 2, TFF3, CALP) with both (carcinogenic parasite) and (parasitic infestation only) and (carcinogenic infection) with different high levels compared to other groups.

Our study was concluded there was relationship between *E. histolytic* and *Giardia lamblia* infection and intestinal cancer .

**Keywords:** Colorectal cancer, mucin 2, TFF3, CALP.

## Introduction

Cancer is a group of diseases involving abnormal cell growth with the potential to invade or spread to other parts of the body<sup>(1)</sup>. These contrast with benign tumors, which do not spread<sup>(2)</sup>. Possible signs and symptoms include a lump, abnormal bleeding, prolonged cough, unexplained weight loss, and a change in bowel movements, While these symptoms may indicate cancer, they can also have other causes, Over 100 types of cancers affect humans<sup>(3)</sup>. Intestinal parasites are one of the most serious health problems in developing countries, especially in tropical and subtropical regions<sup>(4)</sup>. Parasites are a serious public health threat being living organisms that consume their food and other requirements from a host. Medicinal parasites are classified into three main groups: protozoa and helminthes (and some arthropods)<sup>(5)</sup>. This study aims to find out the link between each of the parasites (*E.*

*histolytic* and Giardiasis) infection that causes diarrhea and cancer through some vital indicators that were taken in this study.

## Materials and Method

### Experimental design:

This study contain from 4 groups:

- Diarrhea Parasite and cancer group (G1).
- Diarrhea and cancer without parasites group (G2).
- Diarrhea and Parasite group (G3).
- Control group (G4).

**Samples collection (Stool and Blood):** Stool samples were collected from patients with diarrhea and parasite infections, as well as cancer. And blood

was collected from the same patients in addition to the control group.

**Immunological Examination:** Serum separation -: Blood samples (EDTA-free) were left for 20 minutes at room temperature until the blood clotted, then the samples were centrifuged using a centrifuge at 3000 rpm for 10 minutes, separating the serum from other blood components using a pipette. Min and put into 10 mL tube. Then the tubes containing the serum were preserved at a degree of (-20 ° C) until immunological tests were performed on them. (A) Immunological detection of vital signs: The serum separated by this test is used to estimate the ratio of mucin proteins, trifoyl protein, and calprotectin, using a technique called Enzyme-linked immunoassay (ELISA) technology. immunology assay according to the manufacturer’s instructions (Elabscience).

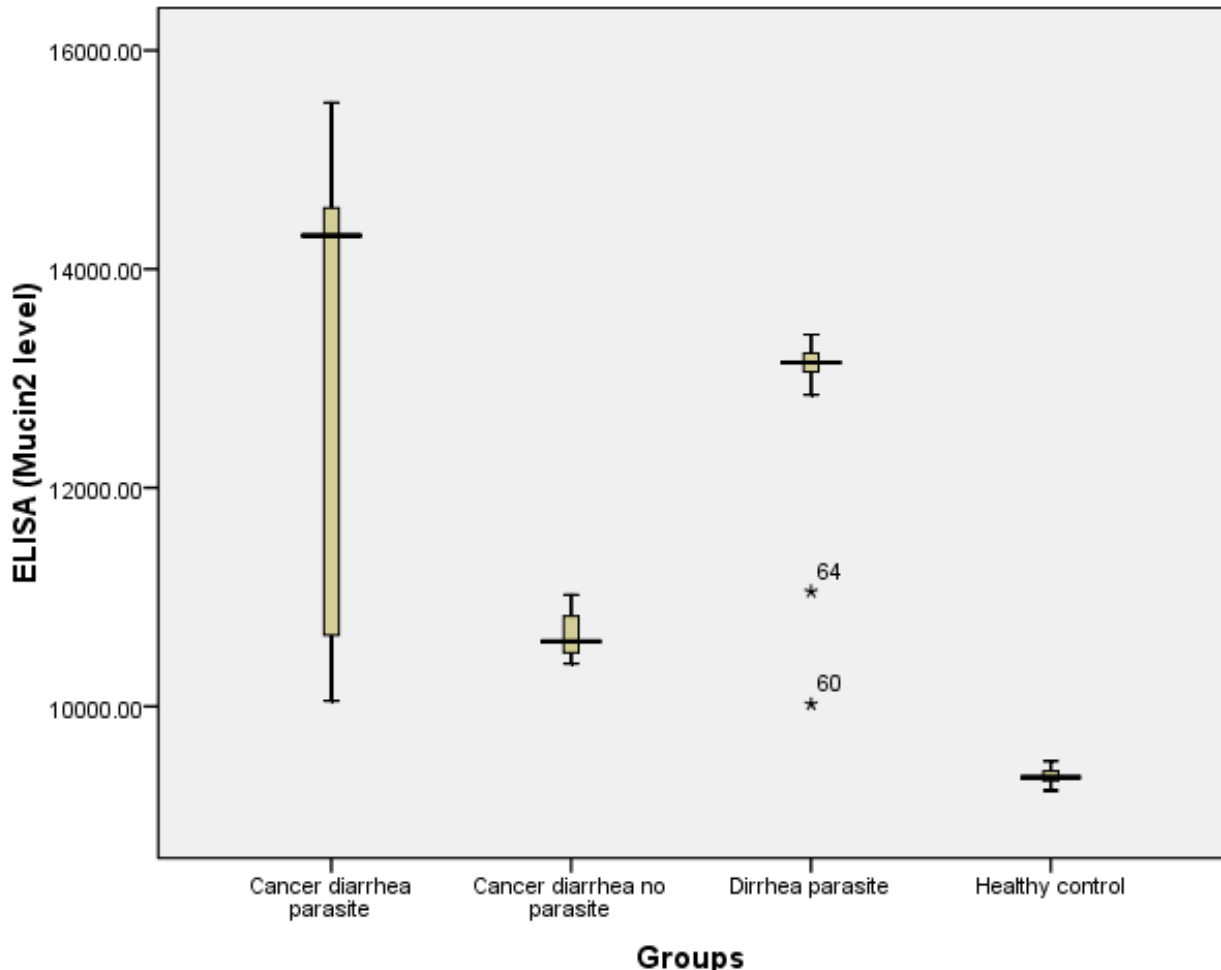
Statistical analysis: - All results in this study were subjected to statistical analysis to find out the significant

differences of the studied variables using the tests (kruskalwallis test) at a probability level( $p < 0.001$ ),<sup>(6)</sup>.

**Kits:** Elisa kits in this study were obtained from the China Origin Company Elabscience.

**Results**

**Mucin (ng/ml):** The results of the study showed that the concentration of mucin 2, the serum levels were 14305.00 (3912.50), 10595.00 (387.50), 13145.00 (175.00) and 9350.00 (90.00) in group of cancer with parasite, cancer with no parasite group, diarrhea and parasite group and healthy control group, respectively. There was highly significant variation in mucin 2 level among study groups ( $p < 0.001$ ). The level was highest in group of cancer and parasite followed by diarrhea parasite group, then by cancer no parasite group and finally by healthy control group, table(1)(4) and figure(1). Thus both cancer and parasite infestation are associated with an increase in the serum level of mucin 2.



**Fig (1):** Box plot shows the average level of mucin serum 2 in study totals.

**Table (1): Measure and Compare Serum Level (Mucin 2) between study groups.**

Characteristic	Cancer diarrhea parasite	Cancer diarrhea no parasite	Diarrhea Parasite	Healthy Control	p
<b>Mucin 2</b>					
Median (IQR)	14305.00 (3912.50)	10595.00 (387.50)	13145.00 (175.00)	9350.00 (90.00)	< 0.001 K HS
Range	10000.00 -15500.00	10400.00 -11000.00	10000.00 -13400.00	9230.00 - 9500.00	

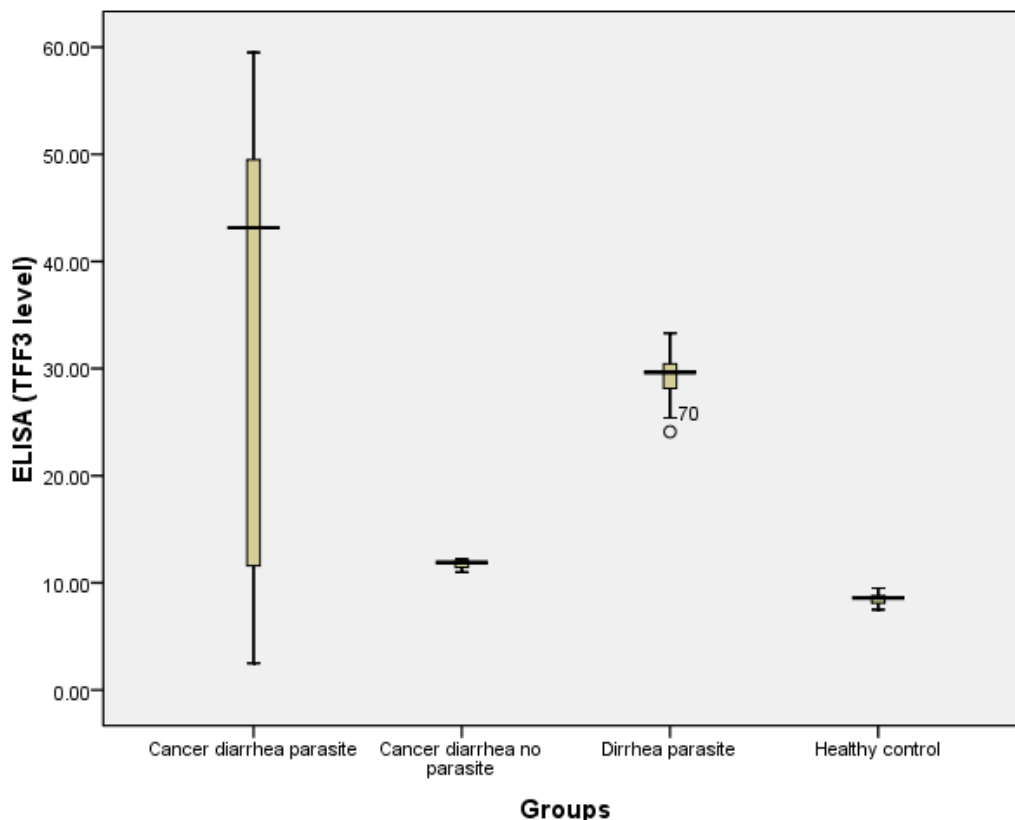
**IQR:** inter-quartile range; **K:** Kruskal Wallis test; **HS:** highly significant at  $p \leq 0.01$

**TFF3 (ng/ml):** The results of the study showed that the concentration of TFF3 TFF3, the serum levels were 43.15 (37.90), 11.90 (0.65), 29.65 (2.45) and 8.60 (0.70) in group of cancer with parasite, cancer with no parasite group, diarrhea and parasite group and healthy control group, respectively. There was highly significant variation in TFF3 level among study groups ( $p < 0.001$ ).

The level was highest in group of cancer and parasite followed by diarrhea parasite group, then by cancer no parasite group and finally by healthy control group, table (2)(4) and figure (2). Thus both cancer and parasite infestation are associated with an increase in the serum level of TFF3.

**Table (2): Measure and Compare Serum Level (TFF3) between study groups.**

Characteristic	Cancer diarrhea parasite	Cancer diarrhea no parasite	Diarrhea Parasite	Healthy control	p
<b>TFF 3</b>					
Median (IQR)	43.15 (37.90)	11.90 (0.65)	29.65 (2.45)	8.60 (0.70)	< 0.001 K HS
Range	2.50 -59.50	11.00 -12.20	24.10 -33.30	7.50-9.50	



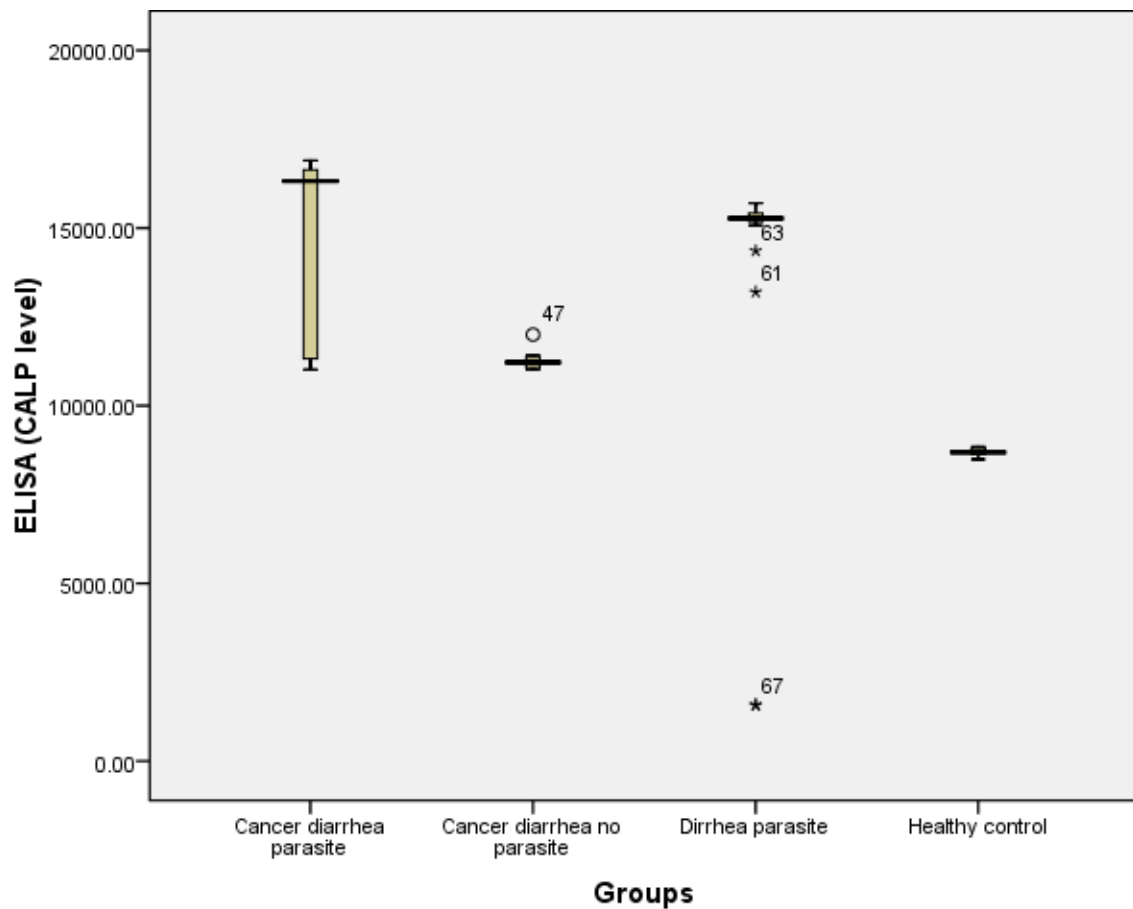
**Fig (2):** Box plot shows the average level of TFF3 serum in study totals.

**CALP ng/ml:** The results of the study showed that the concentration of CALP, the serum levels were 16320.00 (5342.50), 11220.00 (332.50), 15275.00 (240.00) and 8690.00 (170.00) in group of cancer with parasite, cancer with no parasite group, diarrhea and parasite group and healthy control group, respectively. There was highly significant variation in CALP level

among study groups ( $p < 0.001$ ). The level was highest in group of cancer and parasite followed by diarrhea parasite group, then by cancer no parasite group and finally by healthy control group, table (3)(4) and figure(3). Thus both cancer and parasite infestation are associated with an increase in the serum level of CALP.

**Table(3): Measuring and comparing the level of serum (CALP) among study totals.**

Characteristic	Cancer diarrhea parasite	Cancer diarrhea no parasite	Diarrhea Parasite	Healthy control	p
<b>CALP</b>					
Median (IQR)	16320.00 (5342.50)	11220.00 (332.50)	15275.00 (240.00)	8690.00 (170.00)	< 0.001 K HS
Range	11000.00 -16900.00	11000.00 -12000.00	1570.00 -15700.00	8490.00 -8850.00	



**Fig (3): Box plot shows the average level of CALP serum in study totals.**

**Table (4): Concentration of mucin, TFF3 and CAPL in patients with cancer, parasites and diarrhea.**

Characteristic	Cancer diarrhea parasite	Cancer diarrhea no parasite	Diarrhea parasite	Healthy control	Pvalo
<b>Mucin 2</b>					
Median (IQR)	14305.00 (3912.50)	10595.00 (387.50)	13145.00 (175.00)	9350.00 (90.00)	< 0.001 K HS
Range	10000.00 -15500.00	10400.00 -11000.00	10000.00 -13400.00	9230.00 - 9500.00	

Characteristic	Cancer diarrhea parasite	Cancer diarrhea no parasite	Diarrhea parasite	Healthy control	Pvalo
<b>TFF 3</b>					
Median (IQR)	43.15 (37.90)	11.90 (0.65)	29.65 (2.45)	8.60 (0.70)	< 0.001 K HS
Range	2.50 -59.50	11.00 -12.20	24.10 -33.30	7.50-9.50	
<b>CALP</b>					
Median (IQR)	16320.00 (5342.50)	11220.00 (332.50)	15275.00 (240.00)	8690.00 (170.00)	< 0.001 K HS
Range	11000.00 -16900.00	11000.00 -12000.00	1570.00 -15700.00	8490.00 -8850.00	

## Discussion

The results of the study showed a significant increase in the concentration of TFF3 protein in the serum in patients with Parasites and cancer with diarrhea compared to the group of uninfected people and also a significant increase in the group of infected parasite. The reason for the increased concentration of TFF3 in the person with the parasite is its important role in protecting and repairing the mucous layer of the gastrointestinal tract and into cell migration, usually TFF3 increases when the gastrointestinal tract is damaged and in the case of cancer<sup>(7)</sup>. The anomalous expression of trivoyl protein is associated with gastroenteritis and precancerous tumors<sup>(8)</sup>. The results of the current study are consistent with what it did<sup>(9)</sup> where the concentration of TFF3 in serum for people with *Giardia lamblia* parasite increases and the reason is due to the important role of TFF3 in protecting the mucosa and also agrees with the study of<sup>(10)</sup>.

The results of the current study showed a significant increase in the level of Mucinomyocin in the serum in patients with the parasite when compared with the healthy control group. Mucine is considered the type of MUC2 is the main mucous material secreted in the gut. The reason for the high level of mucin in patients with intestinal parasites is due to the important role that myosin plays in protecting the epithelial layer of the intestinal cavity and also works as a barrier to prevent penetration of germs and parasites into the intestinal wall when eating contaminated food and water Parasites hosts the mucous layer of the intestine in which colonies are present<sup>(11)</sup> The presence of bacteria in the mucous layer of the intestine stimulates the immune response of the digestive system and enhances its health<sup>(12)</sup> when invading and colonizing the parasite of the mucous layer of the intestine It stimulates cystic cells in the intestinal cavity from mucus secretion and this reason leads to increased mucus secretion<sup>(13)</sup> as a fungal

defensive state of the gastrointestinal surface layer of the intestine<sup>(14)</sup>. The parasite has a virulence factor that, upon infection, can Stimulating excessive mucus secretion in the intestine and consequently the storage of myosin, which enables it to adhere to the outer mucous layer of the intestine · Myosin plays a protective role through the continuous regeneration of cystic cells and is excreted in the intestinal cavity and does an important job in capturing pathogenic and coexisting parasites and parasites, myosin eventually flows to remove these microorganisms during defecation.<sup>(15)</sup> The current study is consistent with the study of<sup>(16)</sup> Also with the study of<sup>(17)</sup> that showed a high level of MUC2 expression after exposure to the parasite of *Giardia lamblia* also consistent with the study of<sup>(18)</sup> that showed a high level of myosin concentration in parasitic infections and agreed with<sup>(19)</sup> that confirm the high level of myosin in people People with the parasite *Giardia lamblia*, who adopted the reason for the increase to the pathological physiological response of the parasite *Giardia*, which develops disease and diarrhea and works to disrupt the mucous layer of the intestine and impede the absorption process.

The present study found an increase in the serum level of calprotectin in patients with the parasite compared with the control group. Also, there is a slight increase in the serum level of CALP in favor of the infected group compared with the group of infected. This increase may be due to differences in Statistics, sample size and working method can be an increase in the serum level of calprotectin due to intestinal parasites infection. Parasitic infections have a strong effect on the health of the digestive system, especially when the parasite is invasive like *E. histolytica*. This parasite invades the mucous membrane of the intestine and reaches To the epithelial layer which leads to the formation of calculus ulcers<sup>(20)</sup> upon parasitic infection, white blood cells multiply and calprotectin is released,

which activates the action of neutrophils in the intestinal lining, as it participates in the binding and adhesion of white blood cells to the intestinal lining, The effect of infection with the parasite on the blood level in general, especially white blood cells, where the percentage of hemoglobin is low in a person. Injured patients, and this decrease in the percentage of hemoglobin in the blood can be explained by the persistent diarrhea that affects the necessary elements such as iron, copper, and zinc<sup>(21)</sup>. Fecal calprotectin (CF) is considered a vital indicator for the diagnosis of IBD but it is not accurate in the diagnosis, The calprotectin protein is profuse in the leukocytes of neutrophils as it is found in the epithelial cells of the intestine and in the monocytes and monocytes, and the level of calprotectin in the feces is proportional to the level of neutrophil in the gut<sup>(22)</sup>. Mucous membranes increase the liberation of calprotectin and can be found in serum and other body fluids as a clinical inflammatory indication<sup>(23)</sup>. It is due to an increase in white blood cells as an immune response against infection.

### Conclusion

Our study was concluded there was relationship between *E. histolytic* and *Giardia lamblia* infection and colon rectal cancer.

Correlation of elevated serum value of mucin 2, TFF3 and CALP with carcinogenicity, parasite infection and co-infection (parasite with cancer) compared with other indicators.

**Ethical Clearance:** Nil

**Source of Funding:** self

**Conflict of Interest:** Nil

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