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The Complement C3a And C3a Receptor Pathway Indiabetic Patients With Entamobra Histolytica, Cryptospridium Parvum

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Abstract

Cryptosporidium parvum and Entamoeba histolytica are significant protozoan parasites that adversely impact human health. The infection rate of these parasites among diabetic patients was recorded at 18.33%. However, the mechanisms underlying host-parasite interactions remain poorly understood. It was noted that the percentage of E.histolytica was 40%, while that of cryptosporidiosis was 15%, but the interaction between the parasite and the host is not well understood. The results also showed an increase in the C3a/c3aR ratio in diabetic patients infected with intestinal parasites compared to healthy individuals. C3a works by binding to C3aR on the cell surface and has a repair function in intestinal parasite and is important in regulating the immune response. C3a expression levels were analyzed using polymerase chain reaction and compared with the results of Housekeeping gene, which were higher than C3a expression levels, because C3a gene expression is host-specific, it can be influenced by many pathogens in the patient.

Keywords: *Entamobea histolytica , Cryptosporidinm parvum ,C3a , C3aR*

INTRODUCTION

E. histolytica and C. parvum are parasites that cause diarrhea, and individuals vary in their resistance and susceptibility to infection with the disease, especially those with weak immunity [1]. The disease is more severe in children due to their immature immune systems, which are unable to effectively respond to pathogens. Infection occurs through contaminated food and water. C. parvum is found in the small intestine, particularly on the villi. As for E. histolytica, it attacks the intestinal mucosa. The severity of infection and resistance depend on the individual's natural immunity [21] [15] [2] [22]. Type 1 diabetes patients who are insulin dependent are more susceptible to many diseases, especially infections with intestinal parasites [16] [3] [4]. Disorders associated with diabetes patients play an important role in the susceptibility to infection [20] [5]. Complement factor consists of proteins found naturally in blood plasma and produced in the liver a microbe enters the body, When one of the complement proteins is stimulated, it activates another protein and creates a chain of reactions. It has an important role in inflammatory reactions [6]. Complement releases chemicals that attract macrophages to the site of infection and also complements the immune response. The breakdown of complement C3 into its two parts is of great biological importance in immune processes, as C3a stimulates basophils, platelets, and mast cells to release histamine, thus mediating the inflammatory process [9]. C3a contributes to intestinal regeneration via C3aR1-mediated pathways and the expansion of intestinal stem cells. Enterocytes produce C3, which can be cleaved by endogenous cathepsins B and L to generate C3a, Complement contributes to intestinal stem cell function through its binding to C3aR1, and is involved in intestinal regeneration, repair of intestinal parasite, and CD4+ T cell differentiation and development [10].

OBJECTIVE OF THE STUDY: -

1- The role of the complement system in parasitic infections.
2- The effect of diabetes on the immune response.
3- The role of C3a in inflammation and tissue damage.

MATERIALS AND METHODS

A total of 300 samples were collected from patients at Kirkuk General Children's Hospital throughout the year 2024. These samples were analyzed using direct wet mount techniques, Ziehl-Neelsen staining, and light microscopy. Additionally, twenty samples were obtained from healthy individuals to serve as a control group

Blood samples were drawn from diabetic patients experiencing watery diarrhea, abdominal pain, or other gastrointestinal symptoms using sterile syringes. The blood was collected into 15 ml gel-containing tubes and allowed to clot at room temperature. It was then centrifuged at 3000 rpm to separate the serum. A volume of 0.8 ml of the serum was transferred using a sterile pipette into Eppendorf tubes and stored at -18°C until further analysis. ELISA was used to measure C3a and C3aR levels in patients, as per the steps outlined in the (Reed Biotech) manual.

Upon completing the sample collection, the samples were categorized as follows:

In the first group, blood was placed in EDTA tubes for hematological tests, and 1 ml was stored at -18°C for subsequent DNA extraction [17] [23].

The second group had blood prepared with (Trizol) added to it and then placed in a freezer (-18 °C) in preparation for the RNA extraction process. RT-qPCR was performed to measure the level of gene expression using the Transzol Up plus RNA Kit provided by TRANS Company. Statistical analysis used (One Way ANOVA) and t-test to analyze the results and the results were fixed in the form of (Mean ± Standard Deviation).

RESULTS AND DISCUSSION

The current study showed that the overall infection rate was 18.33% for C. parvum and E. histolyica. The increased incidence of these parasites in type 1 diabetes patients is explained by their weakened immune systems, as there is a link between diabetic gastroenteritis and intestinal parasitic infections. Consequently, routine screening for intestinal parasitic infections in diabetic individuals is recommended to prevent further transmission. The high infection rates observed may also be associated with limited health awareness, frequent exposure to agricultural environments, and regular contact with infected animals [11].

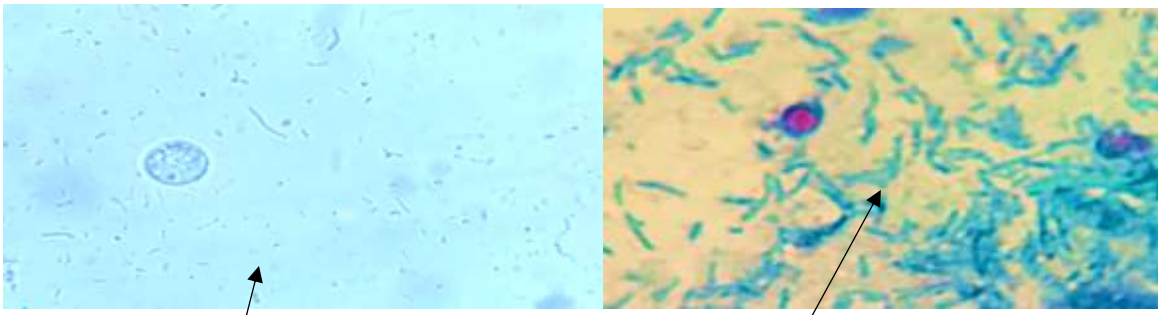

Image (1) The cyst stage of Entamoeba histolytica **Image (2) The cystic stage of the cryptosporidium parvum**

Table (1) The percentage of infection with Entamobea histolytica , cryptosporidium parvum in children with type 1 diabetes

Number of stool samples examined	Positive samples / diabetes	Negative samples

	Number	Percentage%	Number	Percentage%
300	55	%18.33	245	%81.67

As presented in Table (2), the results showed an elevated level of C3a in diabetic patients infected with C. parvum, reaching 3.00849 ± 0.717095. In diabetic patients infected with E. histolytica, the C3a level was 2.03598 ± 0.379250. In contrast, the C3a concentration in non-infected diabetic patients was 0.22278 ± 0.030556, while the healthy control group recorded a value of 0.196990 ± 0.009479.

The complement system plays a vital role as a mediator of acute infections in multiple organs, including the intestine, heart, kidneys, liver, and lungs. Specifically, C3a, a key pro-inflammatory fragment, is involved in regulating intestinal stem cell activity and promoting the formation of intestinal organoids through its interaction with the C3a receptor (C3aR1). It supports intestinal regeneration via C3aR1-mediated mechanisms and by stimulating the proliferation of intestinal stem cells.

Enterocytes produce the complement component C3, which can be cleaved by endogenous cathepsins B and L to generate C3a. Once formed, C3a binds to its receptor (C3aR) on the surface of various cells, contributing to inflammation, intestinal tissue repair, and the differentiation and development of CD4+ T cells. Overall, the host's complement system represents the frontline defense against parasitic infections. As a crucial component of innate immunity, it is activated by a robust proteolytic cascade that leads to the elimination and lysis of invading pathogens. Furthermore, it bridges innate and adaptive immunity by triggering inflammatory responses through the production of pro-inflammatory mediators [12].

Table (2) C3a concentration in the studied samples

Studied samples	Number	Mean ± Std. Error	Range
		Patients	
C. parvum / diabetes	15	a3.00849 ± 0.717095	0.0833-7.6878
E. histolytica / diabetes	40	ab2.03598 ± 0.379250	0.0968-7.7230
Diabetes	20	c0.22278 ± 0 .030556	0.1036- 0.5889
Healthy	20	c0.19699 ± 0.009479	0.1165-0.2423

Similar no significant difference **P= 0.014**
letters indicate

As shown in Table (3), the level of C3aR was significantly elevated in type 1 diabetic patients infected with Cryptosporidium parvum, reaching 201.32088 ± 40.511339. Similarly, diabetic patients infected with Entamoeba histolytica exhibited increased C3aR levels, recorded at 120.95163 ± 15.344666. In comparison, C3aR levels were lower in uninfected diabetic patients and even lower in the healthy control group.

Rapid regeneration of the intestinal epithelium is critical following extensive damage to the intestinal villi caused by parasitic infection. A prolonged disruption of the epithelial barrier can lead to the uncontrolled passage of harmful substances and pathogens into the body. The C3a/C3aR signaling pathway plays a central role in orchestrating this regenerative process [13].

Table (3) C3a R concentration in the studied samples

Studied samples	Number	Mean ± Std. Error	Range
		Patients	
C. parvum / diabetes	15	a201.32088 ± 40.511339	48.376 - 563.49
E. histolytica / diabetes	40	bc120.95163 ± 15.344666	35.8840 - 369.9110
Diabetes	20	c 57.44323 ± 2.289391	39.0169 - 71.8643
Healthy	20	c 54.92510 ± 2.750654	39.0017 - 81.076

Similar letters indicate no significant difference. **P= 0.000**

Table (4) demonstrates that the expression level of the housekeeping gene was higher than that of the C3a Cq gene. This difference arises because the Cq gene is associated with specific host functions and may be influenced by infections or interactions with pathogens, whereas housekeeping genes are fundamental and stably expressed in all cells regardless of external conditions.

The gene expression of C3a reflects the transcription of mRNA followed by its translation into the C3a protein. This expression is tightly regulated to ensure that C3a is produced only when necessary, such as during infection or inflammatory responses. The gene encoding C3a is part of the broader C3 gene, which is primarily expressed in the liver but can also be expressed in other cells, including macrophages and epithelial cells.

C3a plays a key role in the immune response to intestinal parasitic infections by promoting localized inflammation, attracting immune cells, and facilitating tissue repair. However, overactivation of C3a can lead to intestinal tissue damage due to excessive inflammatory responses [14].

Table (4) Measurement of gene expression of C3a gene with reference gene B-Actin

	Case	Mean	Std. Deviation	Std. Error Mean
C3a	Cq Gene	29.2471	1.81472	0.44014
	CT H.K Gene	30.1018	1.98116	0.48050

t = -1.312 df= 32 P value=0.199

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