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Gene sequences of TNF and INFα Cytokines in Cryptosporidium Co-infection with H-pylori of irritable bowel syndrome

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Abstract

In this study, 100 whole blood samples were collected from patients having Cryptosporidiosis and co-infected with H pylori bacteria who attended Baghdad Teaching Hospital during the period from June 2022 to April 2023. The results showed that the Mean±S.E of anti-Cryptosporidium IgM antibodies in the patient group was (14.614±0.901) when compared with the control group (0.081±0.022), with a highly significant difference p<0.0001. Also, the Mean±S.E of anti-Cryptosporidium IgG antibodies was (24.213±0.839) in the patient group when compared with the control group (4.775±0.249), with a highly significant difference p<0.0001. The Mean±S.E of anti-H pylori IgM antibodies in the patient group was (13.923±1.005) when compared with the control group (0.200±0.032), with a highly significant difference p<0.0001. The results also demonstrated that the Mean±S.E of anti-H pylori IgG in the patient group was (28.460±0.847) when compared with the control group (15.835±0.438), with a highly significant difference p<0.0001. The result showed that Mean±S.E of INFα in the patient group was (14.588±0.849) when compared with the control group (4.655±0.271), with a highly significant difference p<0.0001. Data in our study also showed a mutation occurred with TNF gene ID 90865 in SNPs, rs7044343. The variation of wild TT was changed to CC, CC, TC, TC, CC, TC, CC, TC, TT, TC in comparison with the control group. A mutation occurred with INFα gene ID 90865 in SNPs, rs7044343. The variation of wild TT was changed to CC, CC, TC, TC, CC, TC, CC, TC, TT, TT compared to the control group.

Keywords: Gene sequences, TNF, INFα, Cryptosporidium, Co-infection, H-pylori.

Introduction

The protozoan cryptosporidium causes infection in a wide range of vertebrates, such as humans, and can cause acute gastroenteritis. Infected patients present with diarrhea and abdominal pain similar to the manifestations of cholera [1]. The cryptosporidium parasite may cause prolonged fatal infections in an immunocompromised host. Thus, cryptosporidiosis is regarded as one of the riskiest opportunistic diseases that infect AIDS patients [2]. Starting specific and sensitive diagnostic investigations for morbidity reductions and epidemiological surveillance is the best manner for infection control in such patients. At this point, we have summarized the common features of cryptosporidiosis concentrating on the obtainable diagnostic methods utilized for cryptosporidiosis diagnosis [3]. Watery diarrhea is often produced by cryptosporidiosis, although in certain patients the infection may not cause the symptoms. Cryptosporidiosis may be

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underestimated because diarrhea often disappears without treatment [4]. People who are in direct contact with infected animals (especially calves) or drink untreated water or pool water are at a high risk of contracting the infection, although other persons who are not directly in contact with animals can be infected [5]. Cryptosporidiosis is also more frequent in persons with bad health or persons with weakened immune system (such as patients with human immunodeficiency virus HIV/ AIDS, cancers, and transplant patients) [5,6]. Cryptosporidium infection caused more than 50,000 deaths among the parasites which led to nearly one million deaths per year [6]. Following infections, the protozoan changes the intestinal barrier's functions, leading to an increase in its absorption, and permeability with fluid and electrolyte secretions, therefore, the persistence, severity, and outcomes of cryptosporidiosis rely on the immunocompromized status degree [7]. It is well known that intestinal parasites including *Helicobacter pylori (H. pylori)* are highly prevalent in children. Both of these microorganisms can infect the gastrointestinal tracts with overlapping clinical features [8,9]. Patients are colonized with about 27.4% co-existence with H. pylori and significantly correlated with Cryptosporidium species [10]. The significant tumor necrosis factor-alpha (TNF-α) is a pleiotropic cytokine that contributes to inflammations, host defenses, and apoptosis, and plays a double role in immune response regulation, acting as a pro-inflammatory mediator, starting strong inflammatory responses, and as an immunosuppressive mediator, that inhibits autoimmune disease development with tumor genesis [11,12]. Former studies demonstrated that levels of serum cytokines (IL1, & IL-6, and TNF-α) are significantly elevated in patients with gallstones compared to the anti-inflammatory cytokine IL-4 levels in the sera of gallstone patients revealed a significant decrease compared to the healthy control group [13]. Previous studies on the association between gallstones and Helicobacter pylori were not established. Helicobacter pylori are Gram (-ve) and microaerophillic which can cause gastric & duodenal ulcers, chronic gastritis, pancreatic and gastric adenocarcinoma as well as lymphoma of gastric mucosa-related lymphoid tissues [14]. The induced Helicobacter pylori related TNF-α is concentrated in gastric mucosa and it causes no important changes in its levels. Hence, Helicobacter pylori through inducing some inflammatory cytokines, but not IL-10 can be involved in the development of the disease [15]. Our study aimed to detect TNF and INFα in Cryptosporidium Co-infection with H-pylori with irritable bowel syndrome.

Materials and methods

In this study, 100 whole blood samples were collected from patients having Cryptosporidiosis and co-infected with H pylori bacteria who attended Baghdad Teaching Hospital during the period from June 2022 to April 2023. The samples were divided into 2 parts, the first part was centrifuged, and serum was separated for serological tests, and the second part of the whole blood was used for molecular techniques. Cryptosporidium IgM and IgG were measured by sandwich enzyme-linked immunosorbent assay (ELISA) technique. The Helicobacter pylori IgM antibody was measured by the enzyme immunoassay (EIA) technique. TNF-alpha was measured by the Sandwich-ELISA method.

Molecular diagnosis by PCR technique, the primer used:

TNF-F: rs2430561-F TGTAAAACGACGGCCAGTCGTTGCTCACTGGGATTT

TNF-R CAGGAAACAGCTATGACCATGTCTTCCTTGATGGTCTC for IL-6.

IFNα-F TGTAAAACGACGGCCAGTCAGTGAAACAGTGGTGAAGA INFα -R.

CAGGAAACAGCTATGACCTTGTGGAGAAGG AGTTCATAG For INFϒ.

The gene sequence was done by a signer sequencer.

Ethical approval

Before beginning this study, all participants provided written consent. Baghdad Teaching Hospital ethics committee approved the study on number 123/ 318 on June 6, 2022

Statistical analysis

For data analysis, the SPSS-20 program (Faculty version) was used including Mean ± SD with t-test. The ($P < 0.05$) value is considered as significant.

Results

The results showed that the Mean±S.E of anti-Cryptosporidium IgM antibodies in the patient group was (14.614±0.901) when compared with the control group (0.081±0.022), with a highly significant difference $p<0.0001$. Also, the Mean±S.E of anti-Cryptosporidium IgG antibodies was (24.213±0.839) in the patient group when compared with the control group (4.775±0.249), with a highly significant difference $p<0.0001$. The Mean±S.E of anti-H pylori IgM antibodies in the patient group was (13.923±1.005) when compared with the control group (0.200±0.032), with a highly significant difference $p<0.0001$. The results also demonstrated that the Mean±S.E of anti-H pylori IgG in the patient group was (28.460±0.847) when compared with the control group (15.835±0.438), with a highly significant difference $p<0.0001$ as shown in table (1).

Table (1): Mean ±S. E of anti-Cryptosporidium antibodies and H-pylori anti-Hpylori antibodies Co-Co-infections

Parameters	Groups	Mean±S.E	P value
Crypto IgM	**Control**	0.081±0.022	<0.0001**
	Patient	14.614±0.901	
Crypto IgG	**Control**	4.775±0.249	
	Patient	24.213±0.839	
H pylori IgM	**Control**	0.200±0.032	
	Patients	13.923±1.005	
H pylori IgG	**Control**	15.835±0.438	
	Patients	28.460±0.847	

The Mean±S.E of TNF in the patient group was (14.614±0.901) as compared with the control group (0.081±0.022), with a highly significant difference p<0.0001. The result showed that Mean±S.E of INFα in the patient group was (14.588±0.849) when compared with the control group (4.655±0.271), with a highly significant difference p<0.0001 as shown in table (2).

In acute infection with H pylori, the Mean±S.E of H pylori patients was (13.498±0.965a), while in Co-infection patients was (9.077±2.085) when compared with the control group (0.081±0.022), with a highly significant difference between them p<0.0001, as shown in table (3).

In acute Cryptosporidium infection, the Mean ±S. E of H pylori patients was (2.289±0.839a), while in Co-infection patients was (2.289±0.839[a]) when compared with the control group (0.111±0.024), with a highly significant difference between them p<0.0001, as shown in table (4).

Table (2): Mean ±S. E of anti-TNF antibodies and anti-INFα antibodies Co-infections

Parameters	Groups	Mean±S.E	P value
TNF	**Control**	0.081±0.022	**<0.0001****
	Patient	14.614±0.901	
INFα	**Control**	4.655±0.271	
	Patients	14.588±0.849	

Table (3): Mean ±S.E of anti- H pylori IgM antibodies between co-infection patients & H pylori patients

Parameter	Groups	Mean±S.E	P value
H pylori IgM	**Control**	0.081±0.022	<0.0001**
	H pylori Patient	13.498±0.965[a]	
	Co-infection patients	9.077±2.085	
	Between co-infection patients & H pylori patients		0.002**

Table (4): Mean ±S. E of anti-Crypto IgM antibodies between co-infection patients & H pylori patients

Parameter	Groups	Mean±S.E	P value
Crypto IgM	**Control**	0.111±0.024	<0.0001**
	H pylori Patient	2.289±0.839 [a]	
	Co-infection patients	2.289±0.839 [a]	
	Between co-infection patients & Crypto patients		<0.0001**

In chronic Cryptosporidium infection, the Mean±S.E of H pylori patients was (0.086±0.016), while in Co-infection patients was (1.350±0.233a) when compared with the control group (0.096±0.022), with a highly significant difference between them p<0.0001, as shown in table (5).

Table (5): Mean ±S. E of anti-Crypto IgG antibodies between co-infection patients and H pylori patients

Parameter	Groups	Mean±S.E	P value
Crypto IgG	**Control**	0.096±0.022	<0.0001**
	H pylori Patient	0.086±0.016	
	Co-infection patients	1.350±0.233[a]	
	Between co-infection patients & Crypto patients		<0.0001**

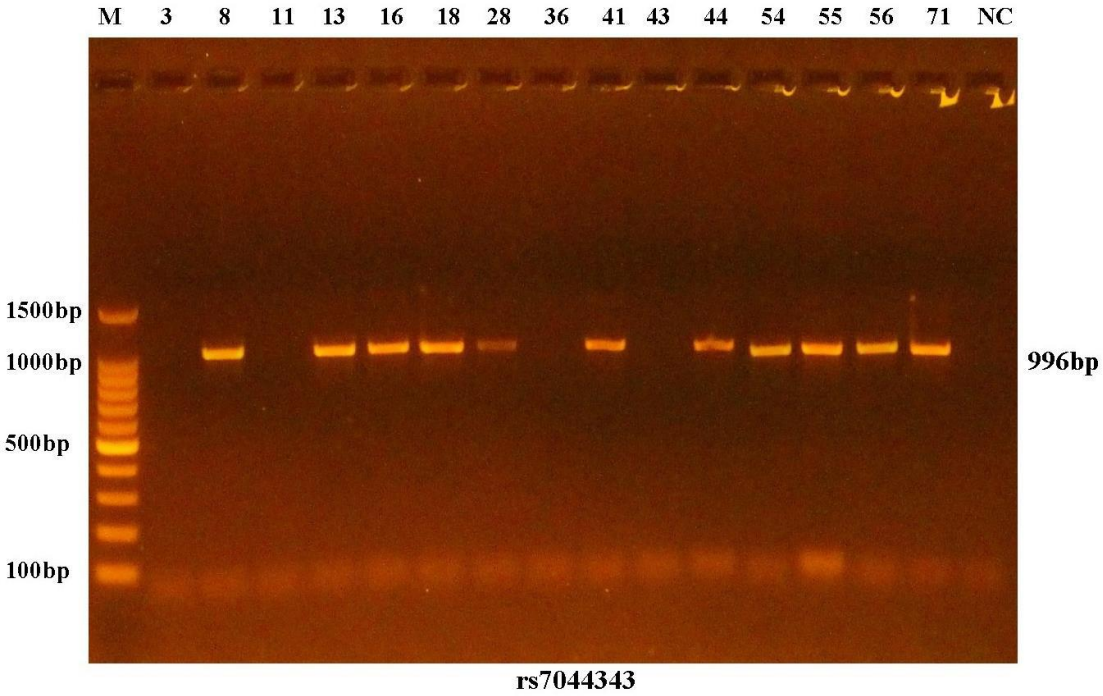

Figure (1): Results of TNF-specific region amplification in Human sample species are fractionated in 1.5% agarose gel electrophoresis stained with ethidium bromide. M: 100bp ladder markers. Lanes 3-71 resembling 996bp PCR product.

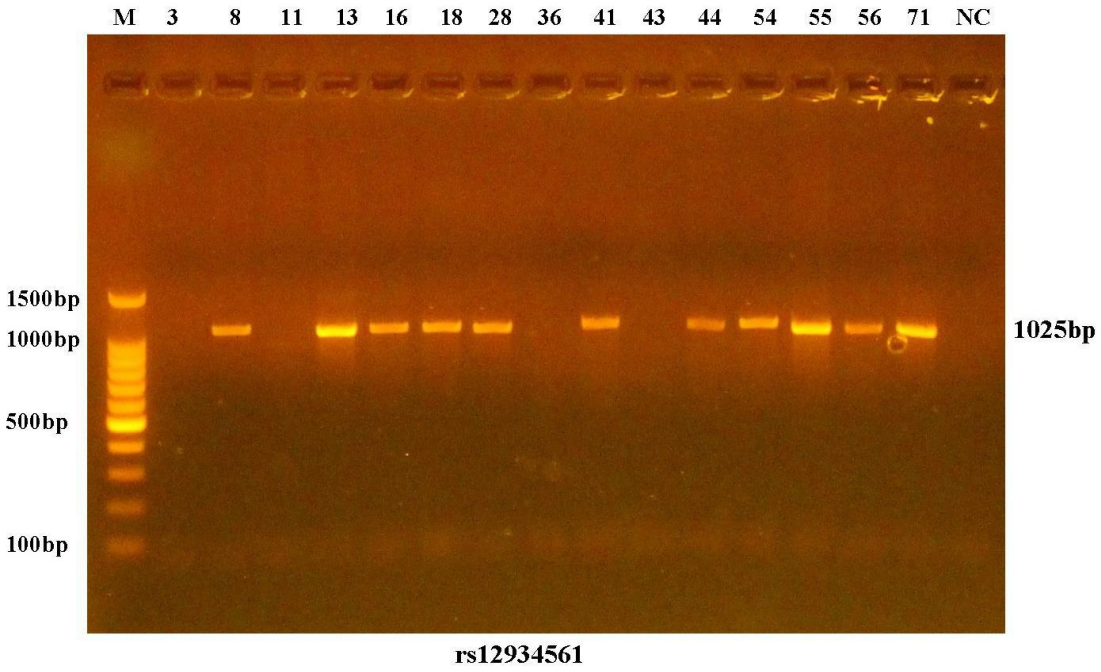

Figure (2): Results of INFα specific region amplification in Human sample species are fractionated in 1.5% agarose gel electrophoresis stained with ethidium bromide M: 100bp ladder markers. Lanes 3-71 resembling 1025bp PCR product.

Table (6) and figure (3) showed a mutation occurred with TNF gene ID 3553 in SNPs, rs1143627. The variation of wild TT and Cc was changed to CC, CC, TT, TC, TC, CC, TT, TC, CC, TC in comparison with the control group.

Table (6): Variation of wild SNPs of TNF gene ID 9235

TNF Gene ID 9235		
SNPs	**rs16944**	**rs1143627**
Wild	**TT**	**CC**
Variation	**T>C**	**C>T**
Samples		
1	TC	TC
2	CC	TT
3	CC	TT
4	TC	CT
5	CC	CC
6	CC	TT
7	CC	TT
8	TC	CT
9	TT	TT
10	TT	TT
C1	TC	CC
C2	CC	TT
C3	CC	TT
C4	TC	CT
C5	CC	TT

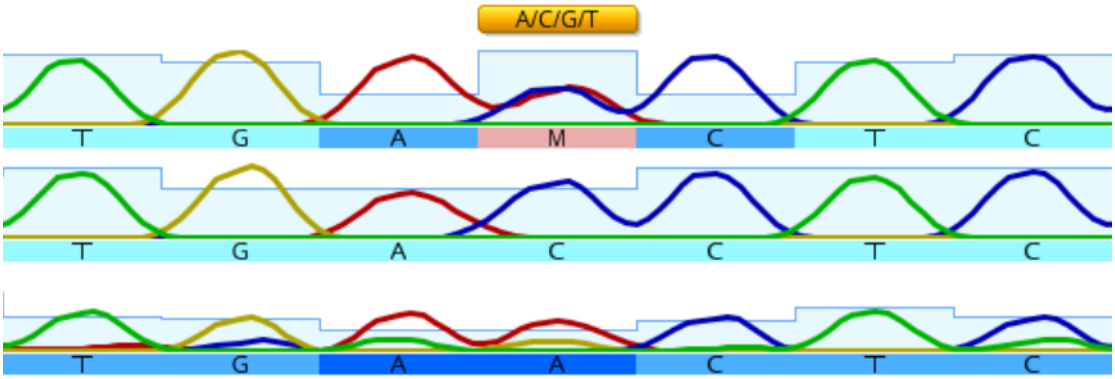

Figure (3): The analysis of rs2283468 SNP of TNF genes by the use of Sanger sequencings. A single "C" peak indicates C homozygous alleles. Single "A" peak indicative of an A homozygous allele. The existence of "C" & "A" peak indicates C/A heterozygous alleles

Table (7) and figure (4) showed a mutation occurred with INFα gene ID 90865 in SNPs, rs7044343. The variation of wild TT was changed to CC, CC, TC, TC, CC, TC, CC, TC, TT, TT in comparison with the control group.

Table (7): Variation of wild SNPs of INFα gene ID 90865

INFα Gene ID 90865	
SNPs	**rs7044343**
Wild	**CC**
Variation	**T>C**
Samples	
1	TC
2	CT
3	CT
4	CT
5	CT
6	TT
7	CT
8	CT
9	TT
10	CT
C1	TC
C2	CC
C3	CC
C4	TC
C5	CC

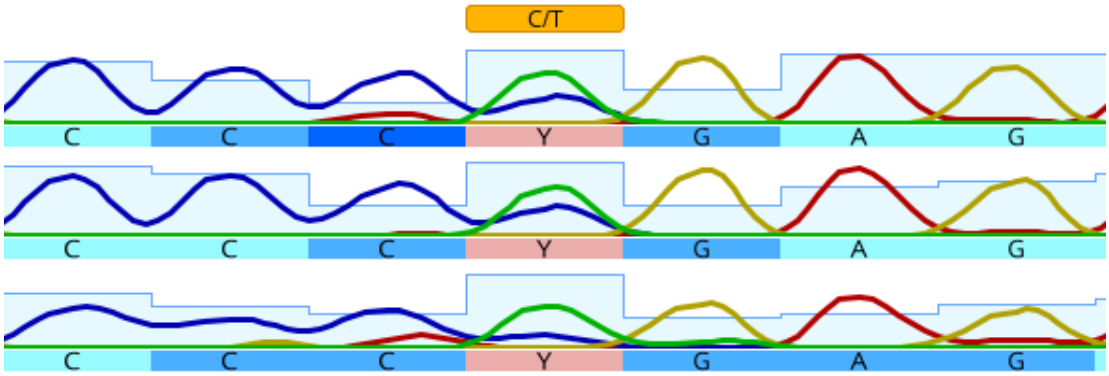

Figure (4): The analysis of rs7044343 SNP of INFα genes by the use of Sanger sequencings. A single "C" peak indicates C homozygous alleles. A single "T" peak indicates T homozygous alleles. The existence of "C" & "T" peak indicates C/T heterozygous alleles

Discussion

According to the results, the Mean ±S.E of anti-Cryptosporidium IgM antibodies in comparison to the control group, there were highly significant differences <0.0001. Naif, et al, (2021) reported the level of anti-Cryptosporedium parvum IgM antibodies, there were highly significant variations in comparison with the healthy individuals in Co-infection among children with Gastroenteritis [16]. Also, there were highly significant differences in the Mean ±S.E of anti-H pylori IgG when compared with the controls (15.835±0.438) p<0.0001. These findings were in agreement with (Urrea-Quezada, et al., 2021) who reported an increase in levels of IgG antibodies in the sera of patients with chronic cryptosporidium infections [17]. The Mean±S.E of TNF antibodies was highly significantly increased in Cryptosporediasis Co-infections with H pylori in comparison to the control group p<0.0001, Akoolo, et al, (2022) concluded there is a very effective role for the tumor necrosis factor-alpha, and there is a significant increase in titration levels in infections common to archaea and bacteria, such as Cryptosporediasis Co-infections with H pylori [18]. With acute H pylori, there were highly significant differences between co-infection patients & H pylori patients p<0.0001. Liu, et al, (2021) demonstrated that the first barriers against H. pylori are gastric epithelial cells. Following infection with H. pylori, it primarily causes stimulation of inflammatory mediator release via the secretion of toxic proteins, ammonia, and nitric oxide to generate toxic impacts on the gastric epithelial cell, leading to damage of the gastric mucosal epithelial with local immunosuppressions, resulting in different gastric case occurrence with Co-infection diseases [19]. With acute Cryptosporidium infection, there were highly significant differences between co-infection H pylori & Cryptosprediasis patients. Ibrahim, et al, (2019) reported that patients are colonized by H. pylori, and was significantly related to Cryptosporidium species with G. intestinalis. The risk of H. pylori existence was higher in January. The H. pylori infection epidemiology is highly correlated with intestinal parasites. The co-existence of H. pylori with Cryptosporidium and G. intestinal could indicate the relationship between H. pylori infection and the fecal exposure markers [8]. While with chronic Cryptosporidium infection, there were highly significant differences between H pylori co-infection patients & Cryptosporidiosis patients. Fadl, et al, (2021) proved that there is a significant increase in chronic co-infections between Cryptosporidium and H. pylori with a high increase

in IgG antibody concentrations among diabetic patients [20]. A mutation occurred with TNF gene ID 90865 in SNPs, rs7044343. The variation of wild TT was changed to CC, CC, TC, TC, CC, TC, CC, TC, TT, TC due to the co-infection of Cryptosporidium and H. pylori. These findings go along with (Carey, et al, 2023, and Mohammed, et al, 2022) who stated that there are multiple mutations on the tumor necrosis factor SNPs resulting from co-infection between the Cryptosporidiosis parasite and bacteria, which deepens the infection and shows its effect on this cytokine [21, 22]. Also, a mutation occurred with INFα gene ID 90865 in SNPs, rs7044343. The variation of wild TT was changed to CC, CC, TC, TC, CC, TC, CC, TC, TT, TT. AL-Eitan, et al, (2021) found that there were a lot of position numbers on INFα SNPs, and multiple mutations occurred along this gene due to the combination of two microorganisms within the human digestive cavity, which often causes stomach ulcers. The pathogenic activity became double actions [23].

Conclusion

According to the results, a mutation occurred with INFα gene ID 90865 in SNPs, rs7044343. The variation of wild TT was changed to CC, CC, TC, TC, CC, TC, CC, TC, TT, TC due to the co-infection of Cryptosporidium and pylori. A mutation occurred with INFα gene ID 90865 in SNPs, rs7044343. The variation of wild TT was changed to CC, CC, TC, TC, CC, TC, CC, TC, TT, TT.

Competing interests

The authors declare that there is no conflict of interest.

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