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RESEARCH ARTICLE

PROTECTION AGAINST THE GIARDIA LAMBILA AND *CRYPTOSPORIDIUM PARVUM* INFECTIONS BY TNF-A, IGA AND IGE

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ABSTRACT

Background: Intestinal parasites are regarded as a serious public health problem. Poverty, illiteracy, poor hygiene, lack of access to potable water, a hot and humid tropical climate is some of the common factors attributed to intestinal parasitic infection. The immune status of the host plays a critical role in determining susceptibility to infection with certain parasite as well as the outcome and severity of giardiasis and cryptosporidiosis.

Objective: To determine the level of TNF-α in serum of parasite infected person also explore the specific IgA and IgE antibody response to *Giardia lamblia* and *Cryptosporidium parvum* infection.

Materials and methods: A total of 142 patients with gastrointestinal complaints randomly selected from central teaching hospital for pediatric in Baghdad (86 females and 56 males) ranging in age from 7 -19 years was collected during 1st April 2012 till 1st August 2013, they were enrolled as a study group and compared with 60 age-gender matched healthy group without gastrointestinal complaints and free of giardiasis and cryptosporidiosis. Patients subjects (61) Giardiasis, (31) Cryptosporidiosis and (50) double infection by stools examination and immunochromatography strips crypto-Giardia. Blood was collected for serum concentrations of TNF-α by used Enzyme linked Immunosorbent Assay (ELISA), while the concentration of, IgA and IgE by auto chemistry analyzer.

Result: The levels of TNF-α ,IgA and IgE in sera patients with *Giardiasis, Cryptosporidiosis and* double infection was increased when compared with the healthy looking controls, the statistical analysis shows significant difference ($P<0.05$) between TNF-α and parasitic infection while not found significant association between infection and levels of IgA and IgE .

Conclusion: Giardiasis, Cryptosporidiosis and double infection had significant effects on TNF-α while non-significant at IgA, IgE levels.

INTRODUCTION

The parasitic infection is a common cause of morbidity and mortality in pediatrics population in tropical countries. The prevalence of intestinal parasites in children varies in different regions of the world [1].

The high prevalence of intestinal parasites in any population is related to parasitic contamination of the soil and water sources in addition to deficient sanitary and sociocultural conditions. The implementation of programmers on health education, personal hygiene, communal sanitation and eventual treatment of infected people would contribute to control of this health problem [2].

Giardia lamblia, Cryptosporidium parvum and Entamoeba histolytica are the most common diarrhea causing parasitic protozoa. Diagnosis of these parasites is usually performed by microscopy. However, microscopy lacks sensitivity and specificity, replacing microscopy with more sensitive and specific nucleic acid based methods [3].

TNF-α is an important inflammatory cytokine in immune regulation and resistance to various microbes including protozoa [4]. TNF-α is a largely produced by macrophages, which can lead to tissue inflammation through the activation of macrophages, recruitment of neutrophils, and up-regulation of other pro-inflammatory mediators [5].

It can also increase cell permeability, resulting in impairment of barrier function and edema formation [6]. The TNF-α plays a central role in mucosal inflammation, and is elevated in the gastrointestinal tract of some forms of inflammatory colitis [7]. Cytokine is known to be of significant importance in the pathogenesis of inflammatory bowel disease (IBD), and anti-TNF agents have proven to effective treatment of some individuals [8].

Immunoglobulin A (IgA) has been implicated in resistance to mucosal infections with bacteria, viruses, and parasites [9].

This study was design to determine the correlation between levels of TNF-α, IgA, IgE and different parameters during parasitic infection.

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SUBJECTS, MATERIALS AND METHODS

Subjects

A prospective study was done on the 142 patients with gastrointestinal complaints randomly selected from central teaching hospital for pediatric in Baghdad (86 females and 56 males) ranging in age from 7 -19 years was collected during April- 2012 and August- 2013, they were enrolled as a study group and compared with 60 age-gender matched healthy group without gastrointestinal complaints and free of giardiasis and cryptosporidiosis

Parasitological Examination

Fresh faecal samples were collected from each patient. They were examined by direct wet mount, Lugol's-iodine stained as well as used the Cer Test Crypto-Giardia card is a one-step colored chromatographic immunoassay (E-50018 Zaragoza-Spain). The results were subjects (61) Giardiasis, (31) Cryptosporidiosis and (50) double infection.

Blood collection

Five ml of venous blood was collected from each patient. The collected sample was transferred immediately in a plain plastic tube and left to clot at room temperature, then spun at 3500 rpm using ordinary centrifuge for 10 minutes, finally the sera were collect and labeled to subsequent analysis. Enzyme linked Immunosorbent Assay (ELISA) used for the detection of TNF- α (Cat No. CSB-E11911h. China) and auto chemistry analyzer was used for IgA (Cat No. 7-202. Poland), IgE (Cat No. 7-231. Poland).

Statistical analysis

Date was analyzed using statistical program for social sciences (SPSS) for windows 11.0 and differences were evaluated using the Chi-Squared test. All tests were two sided with differences considered significant at P<0.05.

Results

Characteristics of study population

A total of 142 stool specimens were examined for the presence of *Giardia lamblia* and /or *Cryptosporidium parvum* of these specimens, 61 were positive for *Giardia lamblia*, 31 positive for *Cryptosporidium parvum* and 50 with double infection. The results of all tests are shown in the table (1)

Table (1) Distribution of study group according to parasite infections

Parasite infection	Number	Percentage	Comparison of Significance Chi2-value	Sig.
G lamblia	61	30.7	Non. Sig.	
C. parvum	31	44.2	(P>0.01)	
Double infection	50	25.1		
Total	142	100%		

The mean age of the patients with gastrointestinal complaints was 14.62±2.9 years when comparing with matched group was 13.54±3.1years. Among patients minimum age was 7 years and maximum 19 years there was significant differences (P<0.05) noticed between both groups.

Significant

According to the age strata highly significant differences noticed between three groups, 16-20 years was constituted the higher percentage (49.3%), while 5-10 years was the lower percentage (14.1%). In the present study it was observed that gastrointestinal complaints percentage was increased with the increasing age, as shows in Table (3).

Out of the 142 patients with gastrointestinal complaints, the frequency of infection was found to be more common among females 86(60.56%) than the males 56(39.43%) as shown in table (4). On the other hand statistical analysis was demonstrated a significant difference in gender distribution.

Significant

Serum levels of TNF-α was summarized in Table 5. In the giardia group, the serum levels of TNF-α was higher than in cryptosporidium and double infection. TNF-α were significantly elevated compared with the levels in control (P<0.01).

Significant

This study was observed the levels of IgA and IgE was increased in studied group when comparing with healthy control group, but statistical analysis shows no significant difference (p>0.05) between both of them as shown in table (6).

DISCUSSION

Protozoan infections of the intestinal tissue will lead to a robust inflammatory process and production of a wide range of cytokines including TNF-α that is elevated in the gastrointestinal tract of some forms of inflammatory colitis, *cryptosporidiosis*, *E. histolytica*-related diarrhea, and *giardiasis* [10, 11].

Table (2) Mean age distribution (years) among the studied groups.

Studied groups	Number	Age/ years			(t-test)/ P-value*
		Mean	Mini	Maxi	
Control	60	13.54±3.1	8	19	(P<0.05)
Patients	142	14.62±2.9	7	19	
Total					

The overall high infection rate with intestinal parasites recorded in the age group of 16-20 years may be due to the fact that this is the age group of secondary school children and working people who are most likely to be exposed to infection through hawker food. In contrast, younger children, especially infants, toddlers and pre-school children would not be exposed too much infection. There were significant differences in the distribution of infection rate among females appeared to be higher than males in this study group, this agreement with finding of Matowicka-Karna *et al*., (2009) and (2011) [12,13].

Table (3) Distribution of patients according to their age strata

Age stratum	Number	Percentage	Comparison of Significance Chi^2-value Sig.
5-10	20	14.1	P<0.0001
11-15	52	36.6	
16-20	70	49.3	
Total	142	100	

This study clearly indicated that the percentage of protozoan parasite-infected and levels of cytokines and immunoglobulin. The serum level of TNF-α was also significantly higher in patients infected with parasites than those uninfected. In addition, tumor necrosis factor-induced nitric oxide production

by macrophages leads to cytotoxicity of *E. histolytica* in vitro [14]. The result of this was in agreement with the finding of Kuhbacher and Fölsch (2007) who reported that the cytokine is known to be of significant importance in the pathogenesis of inflammatory bowel diseases and with finding of Ekhlas *et al*., (2013) who observed these same results.

Table (4) Distribution of intestinal parasites by gender among studied groups.

Parasite infections	Males	Females	Comparison of Significance	
			Chi^2-value	Sig.
Giardia lamblia				
Cryptosporidium parvum	**32**	**29**	Sig.	
	10	**21**	P=0.021*	
Mixed infection	**14**	**36**		
Total	**56**	**86**		

Also agreement with result of Muñoz-Cruz (2010), who strongly suggest that mast cells might be an important source not only of IL-6 but also of TNF-alpha during *Giardia* infection and playing an important role in the outcome of the infection by mast cells mediators generated in response to *G. lamblia* live trophozoites or trophozoite-derived antigens followed by an increase in tryptase expression and a significant release of the preformed mediator histamine. In addition, parasite derived antigens increased TNF-alpha and de novo synthesized cytokine IL-6, at the mRNA and protein level [17]. Zhou *et al.*, Who showed that Tumor necrosis factor alpha (TNF alpha) plays
an important role in the early control of giardiasis [18].

The host defence mechanisms responsible for limiting *Cryptosporidium* infections are not fully understood. However, evidence from several murine, bovine, and human studies suggest an important role for cell-mediated immune responses, including T cell-derived cytokines, in the recovery from *Cryptosporidium infection* [19, 20]. Several studies have reported the induction of a number of cytokines such as IL-1, IL-2, IL-4, IL-5, IL-6, IL-8, IL-15, IFN-γ, TNF-α, TGF-β, and the chemokine rantes following murine, bovine, human, and cell line infections with Cryptosporidium [21, 22]. Of these cytokines, IFN-γ has been shown to be essential in the host protective immune response to *C. parvum* infection.

Table (5) Comparison of serum TNF levels in studied groups.

Groups	TNF-α (pg/ml)	Comparison of Significance (p-value)
G lamblia	30.70±22.42	P=0.02*
C. parvum	25.75±19.57	
Double infection	25.87±15.36	
Control	19.48±14.31	

The current study revealed increased at level of IgA during infection with parasite. Many investigation have documented the IgA has been associated with resistance to a number of mucosal pathogens [23, 24, 25, 26, 27, 28]. Because IgA conferred protection against these mucosal pathogens and Ag-specific IgA responses occur in hosts with cryptosporidiosis, we hypothesized that IgA directed to neutralization-sensitive epitopes may be useful in passive immunization against *C. parvum*.

IgE activates platelets and induces cytotoxic functions against parasites. An elevation in total IgE levels was also reported even in helminth and protozoan infectious diseases such as *cryptosporidiosis* and *giardiasis* [29, 30, 31, and 32].Excretory and secretory proteins released by *G. intestinalis* are responsible for production of a specific IgE [30]. In this study, the serum level of IgE was none significantly elevated in both parasite-infected groups compared to the control. This may be related with limited sample size.

Among the protozoa are *Giardia lamblia*, *Entamoeba histolytica*. *E. dispar*. Parasitic helminth antigens are important to stimulate the production of cytokines such as interleukin-4 and interleukin-5, which act through the induction of IgE synthesis and activation of eosinophils. Eosinophilia is usually detectable in pre-patent period of parasitism, initially linked to B lymphocytes, under the command of Th-2 lymphokines (IL-4 and IL-5), producing IgE in response to initial exposure to an antigen or allergen. Serum IgE high levels occur in tissue migration of larvae or harboring of parasites in tissues [33].

Also other study done by Matowicka-Karna *et al*., (2009) and Jimenez et *al*., (2009) whom revealed that in giardiosis the concentrations of IgE antibodies in blood serum is high [32, 34]. There was a no statistically significant difference occur in levels of IgA and IgE, This result was disagreement with finding of Zarebavani *et al*., (2012).which showed significant correlation

The differences between the results of the previously mentioned studies and even with the results of present study could be related to many factors, like sample size, areas study, methodology and duration of study [35].

In conclusion, parasitic infections are considered the most serious health problems facing the world. Further investigation is needed with large sample size to clarify this issue and studying the role of other cytokines in the protection from parasitic infection

Table (6) levels of IgA and IgE antibodies in serum of studied groups.

Groups	IgA (IU/ml)	IgE (IU/ml)	Comparison of Significance (p-value)	
G lamblia	1.17±0.4	208.6±109.5		.
C. parvum	1.34±0.6	188.8±64.3	(P>0.05)	Non. Sig
Double infection	1.26±0.5	167.2±68.1		
Control	1.32±0.5	186.6±87.2		

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