

Assessment of Serum Interleukin-2, -4 and C-reactive protein Levels in patients with Giardiasis and Cryptosporidiosis

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Summary:

Background: Giardia lamblia and Cryptosporidium parvum are recognized as important health problem in different countries. Humans may infect by two parasites at different age groups. But more common in countries with poor sanitation and unsafe water. Cellular and humoral response is engaged by the host to fight against parasitic infection, cytokines are produced by CD4 of payer's patches or generated from the mucosa lymphoid tissue as result of long duration antigen stimulation via trophozoites or cyst stage or others.

Objectives: To evaluate the levels of IL-2, -4 and C- reactive protein in the sera of patients with Giardia lamblia and Cryptosporidium parvum.

Materials and methods: A total of 142 patients with diarrhea (56 males, 86 females) and compared with 60 healthy looking controls (10 male, 50 female). Attended to the central teaching hospital for pediatric in Baghdad city - Iraq, between 1st April 2012 till 1st August 2013. The diagnosis was established based on direct microscopic examination of stool and Cer Test Crypto-Giardia card, is a one-step colored chromatographic immunoassay. The patients serum had been tested for IL-2, -4 by used Enzyme linked Immunosorbent Assay (ELISA), while the concentration of C- reactive protein was determined by auto chemistry analyzer.

Results: The results of laboratory diagnosis revealed that 61 cases infected with Giardiasis, 31 cases infected with Cryptosporidiosis and 50 cases infected with double infection. levels of IL-2, -4 and CRP in serum patients with Giardia lamblia and Cryptosporidium parvum was increase when comparing with the healthy looking controls, the statistical analysis shows no significant difference ($p < 0.05$) between infected groups.

Conclusion: Giardia lamblia and Cryptosporidium parvum infections had significant effects on IL-2, -4 and CRP levels.

Keywords: Interleukins, C-reactive protein, giardiasis, cryptosporidiosis and inflammatory response.

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Introduction:

Giardiasis and Cryptosporidiosis are two common causes of protozoan diarrheal disease in human [1]. Outbreaks of giardiasis and cryptosporidiosis, which occur via fecal-oral transmission, are associated with consumption of contaminated food, drinking water, daycare centers and recreational water venues [2]. Immunochromatographic assays considered good sensitive and specific methods for rapid diagnosis of Giardia lamblia and Cryptosporidium spp. in human fecal specimens [3]. Intestinal epithelial cells are subject to infect by a range of pathogens, so it seems likely that the immune system needs to discriminate between self and microbial proteins before a specific immune response is made. Little is known of the basis for this discrimination and whether it is made in the draining lymph node or by the dendritic cells (DCs), that transport Ags to the node, Ags ingested by DCs are generally thought to be digested to polypeptides for presentation to T cells, so the recent finding that intestinal nonspecific esterase reaches the mesenteric lymph node (MLN) with intact enzymatic activity

was unexpected. The intestinal epithelial cells from which this nonspecific esterase is derived are engulfed at the end of their life span by DCs in the lamina propria [4]. One of the earliest discovered biomarkers used to diagnose infection is C-reactive protein (CRP) [5]. Which is an acute-phase reactant, and its level measurements are frequently used to aid in the diagnosis of bacterial infections. It Synthesized by the liver and triggered by cytokines (IL-1, IL-6 and TNF- α) and its levels increase within 4-6 hours of an inflammatory stimulus [6]. C-reactive protein produced not only during infection but also in many types of inflammation, it binds to polysaccharides in pathogens, activating the classical complement pathway [7]. Although the role of cytokines in parasitic infections has been widely investigated in animal models, there is limited literature and few clinical works on the importance of Th1 (IL-2) and Th2 (IL-4) in human giardiasis and cryptosporidiosis. Thus, the present work was undertaken to study the levels of these cytokines (IL-2, IL-4 and CRP) in sera of subjects infected with Giardia lamblia and Cryptosporidium parvum and study the correlation with age and gender.

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Patients and Methods:

Sample collections: A total of 142 patients with gastrointestinal complaints including in this study 86 (females) and 56 (males) and majority aged was 7 to 19 years, who attended to the central teaching hospital for pediatric in Baghdad city -Iraq between 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. **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).

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 dispensed in three plastic appendorf tubes, and stored at -20° C until used for serological tests. The patient serum had been tested for IL-2 and IL-4 by using Enzyme linked Immunosorbent Assay for human IL-2 (Cat No. CSB-E04626h- China) and IL-4 (Cat No. DE4435. Germany) while CRP (Cat No. 7-225. Poland) was determined by auto chemistry analyzer.

Statistical analysis: Data 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 and / or Cryptosporidium 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 patients 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	
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±3.1 years. Among patients minimum age was 7 years and maximum 19 years there was significant differences (P<0.05) noticed between both groups. In the present study it was observed that gastrointestinal complaints percentage was increased with the increased age, as shows in Table (2).

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 Total	142	14.62±2.9	7	19	

*Significant

There was a highly significant difference noticed between parasitic infection and age group as shown in table 3, majority of infection was occur in the age group 16-20 years, while the lowest percentage occur at age group 5-10 years.

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	

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 (3).

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

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

In this study it is observed that the levels of IL-2, IL-4 was increased in the studied group when comparing with healthy control group and statistical analysis shows significant difference (p<0.05) between both of them as shown in table (5).

Table (5): comparison of serum cytokine levels in studied groups.

Groups	IL-2 (Pg/ml)	IL-4 (Pg/ml)	Comparison of Significance (p-value)
G lamblia C.parvum	22.49±5.9 21.16±6.5	15.08±6.3 17.61±5.8	P<0.005
Double infection Control	21.88±5.7 12.41±5.6	15.36±6.2 11.59±4.6	

*Significant

Table (6): comparison of serum CRP levels in studied groups

Groups	CRP (mg/dl)	Comparison of Significance (p-value)
G lamblia C. parvum	1.68±0.9 1.280.6±	P<0.005
Double infection Control	1.71±0.7 0.65±0.5	

*Significant

Discussion:

The World Health Organization ranks diarrheal disease as the second most common cause of morbidity and mortality in children in the developing world. Etiological agents of diarrhea are viruses, bacteria, and parasites [8]. Among parasites, Entamoeba histolytica, G.lamblia, and Cryptosporidium spp. are considered to be the most common and important [9]. In the present study used microscopical examination and one-step colored chromatographic immunoassay for detection of parasitic detection in the stool samples due to giardiasis is often hard to diagnosis because of intermittent shedding of organisms [10], requiring examination of stool specimens collected over several day C. parvum may be challenging to detect on modified kinyoun’s acid – fast stained smears due to its small size (4 to 6 µm) [11], and variable staining of the oocysts. Furthermore, microscopic identification requires trained microscopists and involves time and labor for preparing, staining, and examining smears [12], as well as the sensitivity and specificity of the this method [13]. The results of this study indicate that the prevalence of parasitic infection in the females (60.56%) more common than in males (39.43%) this agreement with finding of Matowicka-Karna et al., (2009) and (2011) [14, 15]. Other studies from other countries reported difference in the prevalence of infections between males and females, results were observed by Shariff et al. in 2003 in children under 5 years of age in eastern Nepal found the prevalence of 67.5% of diarrhea in males [16]. Higher prevalence of diarrhea in male was also reported to be 56.4% by Sherchand et al. in Nepal [17], 57% by Eileen J. Klein et al. in Missouri [18] and 61.4% by Sabrina et al. in Tanzania [19]. Higher frequency of diarrhea was seen in age group (16-20) years which was

49.3 % (70/142) than others. This 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 difference in the distribution was not pronounced. Also diarrheal disease remains one of the largest health problems in many parts of the world. And the disease is often mild and self-limiting but, particularly in the elderly and young children, the symptoms may be very severe [20]. Several mechanisms have been proposed to explain the anti-inflammatory effect of parasitic infections, including the production of IL-2, IL-4 and CRP. So this study revealed high levels of cytokines in patients comparing with healthy control group. The percentage of patients producing CRP in blood sample was higher among G. lamblia this agreement with findings of others [22, 23, 24]. Because Giardia is primarily a luminal parasite, the gut-associated lymphoid tissue is likely to play a major role in the immune response [25, 26]. One of the first steps in an effective mucosal response to an infectious agent is the ingestion of the agent and processing and presentation of its antigens by mucosal macrophages [27]. Owen et al.[28] have shown that macrophages from Peyer’s patches have the capacity of engulfing trophozoites of G. muris in vivo. Transmission electron micrographs showed that macrophages with ingested trophozoites were surrounded by lymphocytes, which was reminiscent of rosetting during antigen presentation. Study done by Goyal (2013) indicate oral feeding of LGG to mice for 7 days prior to or simultaneously with the challenge dose of Giardia trophozoites. The administration of the probiotic was continued for 25 days, Giardia seems to have modulated both arms (humoral and cellular) of the mucosal immune system since a significant increase in the levels of specific secretory IgA antibody, IgA+ cells, and CD4+ T lymphocytes were observed in contrast with the decreased percentage of cytotoxic CD8+ T lymphocytes. The stimulated mucosal immune response in probiotic fed Giardia-infected mice was further correlated with the enhanced levels of anti-inflammatory cytokines IL-4,IL-6 and IL-10 and reduced levels of pro-inflammatory cytokine INF-Also study performed by Solaymani-Mohammadi (2011) revealed that infection or other inflammatory insults in the small intestine often result in reduced disaccharidase enzyme levels. Using a mouse model of giardiasis, who examined the role of host immunity and pathogen virulence in mediating disaccharidase deficiency postinfection and found analysis of cytokine production by spleen and mesenteric lymph node cells showed production of IL-4, IL-10, IL-13, IL-17, IL-22, TNF-α, and IFN-γ p.i. with both WB and GS, with IFN-γ being the dominant cytokine for both parasite strains. Mesenteric lymph node cells produced

lower levels of cytokines compared with splenocytes in response to parasite extract, although the overall pattern was similar. Immune responses play a critical role in protection, and resolution of, cryptosporidiosis. However, the nature of these responses, particularly in humans, is not completely understood. Both innate and adaptive immune responses are important. Innate immune responses may be mediated by Toll-like receptor pathways, antimicrobial peptides, prostaglandins, mannose-binding lectin, cytokines and chemokines. Cell-mediated responses, particularly those involving CD4+ T cells and IFN- γ play a dominant role. Mucosal antibody responses may also be involved. Proteins mediating attachment and invasion may serve as putative protective antigens. Further knowledge of human immune responses in cryptosporidiosis is essential in order to develop targeted prophylactic and therapeutic interventions. This review focuses on recent advances and future prospects in the understanding of human immune responses to *Cryptosporidium* infection [31]. Other study done by demonstrated that In the clinical tests, levels of c-reactive protein, erythrocyte sedimentation rates, and neutrophil proportions were normally increased in the peripheral blood, whereas the lymphocyte proportion exhibited a tendency towards decrease. The pathological findings were compatible with an inflammatory reaction in the host c-reactive protein (CRP) levels is high, but not in all cases [32]. Recently it was reported that innate immunity rather than adaptive immunity played a major part in the recovery phase of acute infection in neonatal mice [33]. Adult mice lacking T and B cells develop chronic *C. parvum* infection that is usually fatal, but for several weeks these animals may efficiently control parasite reproduction. The innate immunity involved is at least partly IFN- γ dependent, as infection levels increased after treatment with anti-IFN- γ -neutralizing antibodies [34, 35]. Numerous studies have demonstrated that innate immunity is a major element in host defense against *C. parvum*, and important factors involved in this arm of the immune response include NK cells, IFN- γ , and IL-12 [36, 37, 38, and 39]. 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.

Author's contribution:

Study conception: lecturer Mohammed J. Shakir

Study design: lecturer Mohammed J. Shakir and Dr. Areej A. Hussein

Acquisition of data analysis: lecturer Mohammed J. Shakir and Dr. Areej A. Hussein

Interpretation of data analysis: Dr. Areej A. Hussein

Drafting of manuscript: Dr. Areej A. Hussein

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