C -Reactive Protein as an Immunopathological Prognostic Marker for Giardia Lamblia and Entamoeba Histolytica Associated Diarrhea among Children of Baghdad Governorate

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Abstract

Background: C-reactive protein (CRP) is one of the classic acute phase proteins. The study of induction of acute phase reactants synthesis in parasitic infections would aid in understanding the host –parasite relationship*.***Methods:**Seventy three children with parasitic diarrhea that proved through clinical and microbiological investigation were selected. Thirteen of selected children were infected with *G.lamblia* and the rest sixty were infected with *E.histolytica* . Nineteen of the control group was females while the rest eleven were males. Venous Blood were collected from patients and control group for detection of CRP level via latex agglutination test. **Results :** The high rate of infection was detected among the age group (19-36) months. The highest level of CRP was (192 mg/dl) while the lowest one was (12 mg/dl) with a mean level (51.20 mg/dl). the mean level of CRP in control group was (3.7500 mg/dl). positive significant correlations between CRP level and the age of infected children (r=0.290,p=0.013), the chronic infection with *G.lamblia* and *E.histolytica* (r=0.760,p=0.000) . Positive significant correlation between the age group and the chronic infection with *G.lamblia* and *E.histolytica* (r=0.493,p=0.000) . Significant difference was detected between the patients group and control group regarding the CRP level (p>0.05).**Conclusion:** This study proved that CRP level was increased in association with the age and chronic presentation of diarrhea caused by *G.lamblia and E.histolytica* and can be used as a non specific immunological marker for monitoring of clinical presentation of *G.lamblia and E.histolytica* associated diarrheal .

Key word: CRP, G.lamblia, E.histolytica, diarrhea

1. Introduction:

C-reactive protein (CRP) is one of the classic acute phase proteins.CRP was originally discovered by Tillett and Froncis in 1930as a substance in the serum of patients with acute inflammations that react with C-polysaccharide of pneumococcus [1] . Initially it was thought that CRP might be a pathogenic secretion as it was elevated (100-1000 fold) in the people with a variety of illness such as infections, trauma, surgery, burn and malignant diseases. It's thought to bind to phosphocoline thus initiating recognition and phagocytosis of damaged cells [2]

Measuring and charting of CRP value can prove usefulness in determining disease progress or the effectiveness of treatment [3] .In children living in malaria endemic region elevated CRP level concentration are common .its mean level (7-8 mg/dl) [4]. Plasma CRP level can be valuable for identification of post *kala-azar* dermal leishmaniasis after treatment [5]. Systemic inflammatory reactions are prominent features of many parasitic infections. Cellular and humoral component of acute phase reaction may have an impact on the host –parasite relationship[6].The regulatory function of acute phase reactant on immunological and inflammatory response suggest that the study of induction of acute phase reactants synthesis in parasitic infections would aid in understanding the host –parasite relationship. The parasitic infections lead to the early induction of enhanced synthesis by hepatocytes of all acute phase reactants [7]

Aim of the study: This study try to evaluate the usefulness of C- reactive protein (CRP) level as a prognostic marker for *G.lamblia and E.histolytica* associated diarrhea in children.

2. Materials and Methods:

2.1. Selection of patients and control:

Seventy-three children with parasitic diarrhea that proved through clinical investigation as well as microbiological examinations attended to outpatient's clinic of Baghdad teaching hospital and children care hospital during a period from January 2008 to May 2008 selectively enrolled in this study. The present research was approved by ethic committee of Diyala university, college of medicine. At first the aim of study was explained for all participants or their parents and after obtaining their oral consent they have been studied.

Thirteen of selected children were infected with *G.lamblia* and the rest sixty were infected with *E.histolytica*. Forty-two (57.5%) out of   Seventy three children were males with mean age (43.7± 21.26)

months, on the other hand females represent the rest 31(42.5%) with mean age (33.6 ± 27.72) months. Thirty apparently healthy children were selected as a control group. Nineteen of the control group was females while the rest eleven were males.

2.2. Methods:

A. Detection of parasitic infection:

Stool samples of patients group were submitted for direct microscopical detection of *G.lamblia* and *E.histolytica* through wet preparation technique [8] .Five to ten milliliters of venous Blood were collected from patients as well as control group blood samples were left at room temperature for clotting. Sera were separated by centrifugation at 300 RPM and divided in to aliquots (250 microliters for each one) and stored at -20 c^o until use for detection of CRP level via latex agglutination test using commercial kit from spin react company[9] .

B. CRP-latex agglutination:

Reagents and samples allowed reaching room temperature. About 50 µL of the sample and one drop of each Positive and Negative controls were added into separate circles on the slide test. Then the CRP-latex reagent was mixed vigorously before using and (50 µL) was added next to the samples to be tested. The drops were mixed with a stirrer, spreading them over the entire surface of the circle. the slide was Placed on a mechanical rotator at 80-100 r.p.m. for 2 minutes. False positive results could appear if the test read later than two minutes. The approximate CRP concentration in the patient sample is calculated as follow[9]:

6 x CRP Titer = mg/L

2.3. Statistical analysis:

Data analysis was performed using T-test to find out the significant differences between two groups that composed from continuous variables. Spearman's test (rho) for correlation was used for categorical and non-categorical data. The level of significance was 0.05(two-tail) in all statistical testing; significant of correlations include also 0.01 (two-tail) .The level of confidence limits was 0.095.Statistical analysis was performed using SPSS for windows TM version 14.0. and Microsoft Excel for windows 2007.

3. Results:

This study revealed that minimum age of infected children was 2 months and maximum age 134 months with a mean age 132 months while minimum age of apparently healthy children was 12 months and maximum age 144 months with a mean age 70 months as shown in table(2). The highest rate of infection was detected among the age group (19-36) months that represent (40%) of total infected cases while the age group (91-108)month and (127-144) month represent the lowest age group encountered infection with *G.lamblia* and *E.histolytica* as shown in table (1).Regarding control group ,more frequent age was(19-36)months that represent (30 %) fallowed by (37-54) months, (73-90) months, (109-126) months that represent (6.667)% respectively as shown in table (1).

Table (3) explain that among infected children, the highest level of CRP was (192 mg/dl) while the lowest one was (12 mg/dl) with a mean level (51.2055 mg/dl) compared with control group in which the highest level of CRP was (12 mg/dl) while the lowest one was (6 mg/dl) with a mean level (9.40 mg/dl).significant difference was detected among infected children and control group (p<0.05) regarding CRP level.

This study revealed that there was a positive significant linear relationship between the level of CRP and the age of infected children (r=0.290,p=0.013) on the other hand ,there was no statistically significant linear relationship in the level of CRP was found regarding gender of infected children(r=-0.034, p=0.772)as shown in table (4)

This study proved that there was a positive significant linear relationship between the level of CRP and the chronic infection with *G.lamblia* and *E.histolytica* among children (r=0.760,p=0.000) on the other hand ,there was a negative significant linear relationship between the level of CRP and the acute presentation of diarrhea caused by infection with *G.lamblia* and *E.histolytica* among children (r= - 0.794,p=0.000) as shown in table (4).

This study elucidated that there was a positive significant linear relationship between the age group and the chronic infection with *G.lamblia* and *E.histolytica* among children (r=0.493,p=0.000) on the other hand ,there was a negative significant linear relationship between the age group and the acute presentation of diarrhea caused by infection with *G.lamblia* and *E.histolytica* among children (r=- 0.447,p=0.000) as shown in table (4).

Table (1): Distribution of patients and control group according to age

Age group (months)	Infected children No. (%)	Control group No. (%)
1-18	20 (27.39%)	3(10%)
19-36	29(39.72%)	9(30%)
37-54	14(19.17%)	2(6.66%)
55-72	4(5.47%)	3(10%)
73-90	4(5.47%)	2(6.66%)
91-108	1(1.36%)	5(16.66%)
109-126	0(0%)	2(6.66%)
127-144	1(1.36%)	4(13.33%)
Total	73(100%)	30(100%)

Table (2): Description of age among infected children and control group.

		Range	Minimum	Maximum	Mean	Std. Error	Std. Deviation
Age (months)	Infected Children	130.00	2.00	132.00	34.4521	2.8099	24.0081
	Control group	132.00	12.00	144.00	70.6000	8.0379	44.0255

Table (3): Description of CRP level among G.lamblia & E.histolytica infected children and control group.

Parameter	CRP level (mg/dl)		P- value
	infected children	control group	
Mean± S.D	51.2055±46.4265	9.4000±3.0240	(p<0.05)
Std. Error of Mean	5.4338	0.5521	
Median	48.0000	12.0000	

Std.Deviation

Table (4): correlations among age, gender, CRP level, clinical presentation and causative agent of diarrhea.

Parameters	Spearman's correlation coefficient	CRP Level	Acute infection	Chronic infection	G.lamblia
Age	r	0.290	-0.447	0.493	0.134
	P value	0.0130	0.000	0.000	0.257
Gender	r	-0.034	0.023	-0.047	-0.207
	P value	0.772	0.847	0.694	0.079
CRP level	r		-0.794	0.760	0.104
	P value		0.000	0.000	0.383
Acute infection	r			-0.972	-0.063
	P value			0.000	0.594
E.histolytica	r				-1.000
	P value				.000

4. Discussion:

This study elucidate that highest incidence of *G.lamblia & E.histolytica* associated diarrhea was determined in the age group (1.2-18) and (37-54) months respectively .In the other hand spearman's test for correlation proved that positive significant linear relationship was found between age of infected children and CRP level . Statistical significant difference was found between *G.lamblia & E.histolytica* positive cases and control group regarding CRP level. This may be due to increase the chance of exposure of childrens at this early life stage to *G.lamblia & E.histolytica* through contaminated water or foods as well as behavioral causes related to tendency of childrens to discover the surrounding environment throughout touch and taste behavior that increase the chance of infection with *G.lamblia & E.histolytica*.in addition the susceptibility of infection may

also belongs to the fact that the immune system at this stage of life was immature .Sanitation procedures of foods and drinking water put another building block for catching also play a role in increase susceptibility of infections . Socioeconomical status and educational level of family also play a vital role in increase susceptibility infection with *G.lamblia & E.histolytica* [10, 11]

Negative significant linear relationship was detected between acute infection with *G.lamblia & E.histolytica* and the serum level of *CRP* .this may be due to the fact that acute cases of *G.lamblia & E.histolytica* associated diarrhea are more frequently occur in early childhood during which immature immune system participate in defense against *G.lamblia & E.histolytica* via acute phase reactant including CRP even in small amount from functionally active hepatocytes . On the other hand the activity of hepatocytes will be more organized throughout the time and the amount of CRP will be more than that of acute phase of diarrhea associated by *G.lamblia & E.histolytica* [8] **[10]**. The maturity of immune response also play a role in this negative correlation between the acute phase of diarrhea associated by *G.lamblia & E.histolytica* and the level of CRP ,during this phase the innate immune mechanism recognize pattern recognition molecules on the tegument of infected *G.lamblia & E.histolytica* that lead to increase the activity of mucosal associated macrophages to starting destruction of the parasites that mean get rid of infection during short period of time associated with slight elevation in CRP level [12]. Other possible cause for decreasing CRP level during early acute phase of diarrhea may be associated with site of infection with *G.lamblia & E.histolytica* which is the gut mucosa that may cause masking of parasite pattern recognition molecules that leads to protection from mucosal IgA and IgM as a humoral arm of immune defense mechanism as well as from phagocytic activities of mucosal associated macrophages .The small number of parasitic stages that swallowed actually give another explanation for low level of CRP during acute phase of diarrhea associated with *G.lamblia & E.histolytica infection* [6-8]

This study proved that during chronic phase of *G.lamblia & E.histolytica* associated diarrhea there was positive significant increase in the level of CRP , this may be due to multiple causes one of them may be the large number of *G.lamblia & E.histolytica* infective stages was swallowed ,long duration of infection cause increase in the activity of hepatocytes for increase synthesis of acute phase reactant including CRP .On the other hand tissue damage that caused by attachment of parasite on the mucosal surface that may represent a continuous stimulator for hepatocytes for increase synthesis of CRP as innate defense mechanism that collaborated with increase phagocytic activities of mucosal macrophages as well as mucosal IgA as a humoral defense mechanism against of *G.lamblia and E.histolytica* infective stages [2, 4]

Negative significant linear relationship was detected between *G.lamblia and E.histolytica* infective stages, this may be due to the way of tissue invasion and parasitization that not permit to co infection with both parasite via tissue tropism. Antagonism may be another possible cause of this negative relationship as well as the possibility of production of inhibitors from one parasite to inhibit other one from colonization and infection. Molecular difference in the receptor responsible for mucosal colonization or in other word molecular antagonism at the receptor level may be found and prevent co infection with G.lamblia *and E.histolytica* infective stages [2, 8, 12]

5. Conclusion: this study proved that the age of 1.5 - 4.5 years of children represent the most critical period of parasitic diarrhea caused by *G.lamblia* and *E.histolytica.* CRP level was increased in association with the age and in   chronic presentation of diarrhea caused by *G.lamblia and E.histolytica* and such correlation not present with gender of children .CRP can be used as a non specific immunological marker for monitoring of clinical presentation of diarrheal disease among by *G.lamblia and E.histolytica* infected children.

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