



Serum level of Interleukin (IL)-8, Monocyte Chemoattractant Protein (MCP)-1 and Tumor Necrosis Factor (TNF)- α in children infected with *Entamoeba histolytica*

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ABSTRACT

E. histolytica is an enteric protozoan parasite which caused tissue invasive amoebiasis. It can induce the production of cytokines (such as IL-8 and TNF- α) and chemokines (such as MCP-1) from intestinal epithelial cells. This study was done to detect the serum level of IL-8, MCP-1 and TNF- α , using an enzyme-linked immunosorbent assay, in 60 children affected by amoebiasis to correlate the production of these cytokines with infection and comparison with the healthy persons (n= 30). Three aged groups (1 month- 2 year, 2-4 year and 4-6 year) were entered in the study for patients and healthy controls. The level of IL-8 (37.22 ± 7.06 , 37.35 ± 1.981 and 36.45 ± 4.39 pg/ml) in children with amoebiasis were higher significantly compared with the level in healthy children (29.95 ± 4.30 , 26.90 ± 4.17 and 25.32 ± 2.31 pg/ml) in all age groups (1 month- 2 year, 2-4 year and 4-6 year, respectively). Significant differences in the level of MCP-1 which were higher (28.92 ± 1.48 , 30.85 ± 3.78 and 31.91 ± 6.82 pg/ml) in children with amoebiasis than in healthy children (18.44 ± 0.74 , 22.62 ± 6.25 and 18.30 ± 1.43 pg/ml) in all age groups (respectively). Also, the level of TNF- α were higher significantly (58.05 ± 6.90 , 55.87 ± 3.81 and 57.32 ± 8.86 pg/ml) in children with amoebiasis compared with healthy children (42.91 ± 2.54 , 43.54 ± 3.42 and 42.64 ± 3.47 pg/ml, respectively) in all age groups (respectively). These findings reflect a role of IL-8, MCP-1 and TNF- α in *E. histolytica* infection.

1. Introduction

Entamoeba histolytica is an enteric protozoan parasite of human and the causative agent of invasive amoebiasis [1]. It has worldwide distribution, and it is considered as health risk in most of countries [2]. *E. histolytica* are endemic in tropical and subtropical regions, it is causing the dysentery and liver abscess in the most cases. Infected persons suffer from wide range of disease severity, according to the immunity of patient and his nutritional status, the infective dose and the pathogenic potential of the infecting organism [3].

Pathogenesis of intestinal amoebiasis may be related with the inflammatory responses of the host [4]. The results of research on inflammatory host response during amoebiasis has explain that *E. histolytica* can stimulate the production of cytokines and chemokines from intestinal epithelial cells [5]. These studies [6, 7, 8] established the ability of *E. histolytica* to induce

the production of inflammatory cytokine (such as Interleukin (IL)-8) from epithelial cells.

Monocyte chemotactic protein (MCP)-1 is one of chemokines which many types of cells produced it, including intestinal epithelial cells. It has great ability to chemotactic the monocytes, lymphocytes and basophils [9]. Kammanadiminti *et al.* [4] refer to the role of colonic epithelial cells in the initiation of inflammation by secreting MCP-1 in the response to soluble substances secreted from *E. histolytica*.

Both of enterocytes and macrophages can secreted Tumor necrosis factor (TNF)- α which is a component participate in the amplification of the inflammation and it is related with the intestinal damage during amoebic infection [10].

The present study undertaken to determine the level of IL-8, MCP-1 and TNF- α in the sera of infected

children with *E. histolytica* in Salah Al-Din province, Iraq.

2. Materials and Methods

2.1. Study samples:

During the period from July till December 2017, the present study is done on the patients of General hospital of Tikrit and Samarra city at Salah Al-Din province, Iraq.

A total of 60 human stool samples were collected from patients infected with *E. histolytica*. Diagnosis of infection was based on the microscopic examination of stool and confirmed the stages (trophozoite or cyst) of parasite in stool smears which done by using saline and iodine wet-mount method [11].

For serum isolation, whole blood has been collected from patients in a test tube free from anticoagulants and leave it 20 minutes (at room temperature) for clotting. Clot has been then removed by centrifuging at $1500\times g$ for 10 minutes in a cooled centrifuge. The resulting supernatant was designated serum [12]. The serum has been immediately transferred into a clean eppendorf tube using a Pasteur pipette. The samples were kept at -2°C till handling to analyzed, with avoiding repeating of freeze-thaw the serum to prevent the destructive effect on its components.

Healthy controls ($n=30$) were selected randomly from children of Tikrit and Samarra city. Decision was made whether the control groups were in healthy status or not according to the apparent healthy of person with no history of current disease and pathological status.

Three aged groups (1 month- 2 year, 2-4 year and 4-6 year) were entered in the study for patients and healthy controls.

2.2. Cytokines detections

Serum IL-8, MCP-1 and TNF- α level were measured by enzyme-linked immunosorbent assay (ELISA) technique (kits, Shanghai, China). The procedure of technique was done by following the manufactures' instructions. At the end of experiment, the values were expressed as mean optical densities at 450 nm. Standard curve for different standard concentrations had been drawing with their absorbance. Then each concentration (pg/ml) of cytokines was calculated and then evaluated statically.

2.3. Statistical analyses

All data were analyzed using SPSS (statistical package for social science, version 10.0) computerized program. All results were given as mean $\pm$ standard error (SE) followed by range. F-test was used for comparison between mean values of groups. The statistical significance was accepted as P value < 0.05 .

3. Results

The results showed significant differences in the level of IL-8, MCP-1 and TNF- α in serum when compared between infected and uninfected children in all of age group.

The level of IL-8 (37.22 ± 7.06 , 37.35 ± 1.981 and 36.45 ± 4.39 pg/ml) in children with amoebiasis were higher compared with the level in healthy children (29.95 ± 4.30 , 26.90 ± 4.17 and 25.32 ± 2.31 pg/ml) in all age groups (1 month- 2 year, 2-4 year and 4-6 year, respectively). Also, the level of TNF- α were higher (58.05 ± 6.90 , 55.87 ± 3.81 and 57.32 ± 8.86 pg/ml) in children with amoebiasis compared with healthy children (42.91 ± 2.54 , 43.54 ± 3.42 and 42.64 ± 3.47 pg/ml, respectively) in all age groups (respectively). The level of MCP-1 were higher (28.92 ± 1.48 , 30.85 ± 3.78 and 31.91 ± 6.82 pg/ml) in children with amoebiasis than in healthy children (18.44 ± 0.74 , 22.62 ± 6.25 and 18.30 ± 1.43 pg/ml) in all age groups (respectively).

No significant differences in the level of IL-8 and TNF- α were recorded when compared between infected children or uninfected children of age groups. Also, no significant differences in the level of MCP-1 were recorded in the comparison between the infected children in age group 1 month- 2 year with age group 2-4 year and 4-6 year. The differences in the level of MCP-1 were not significantly when compared between the uninfected children in age group 1 month- 2 year with age group 4-6 year, whereas significant differences in the level of MCP-1 were recorded when compared between uninfected children in age group 1 month- 2 year with age group 2-4 year.

The mean level and rang of IL-8, MCP-1 and TNF- α were shown in tables (1, 2 and 3, respectively).

Table (1): Comparison of serum IL-8 level in patients infected with *E. histolytica* and in healthy controls.

Groups		No. samples	Serum level of IL-8 (pg/ml)	
			(Mean $\pm$ SE)	Range
1 month- 2 year	Infected	28	37.22 ± 7.06 a	31.30- 59.55
	Uninfected	10	29.95 ± 4.30 b	24.66 - 37.53
2-4 year	Infected	12	37.35 ± 1.981 a	34.66 - 40.40
	Uninfected	10	26.90 ± 4.17 b	23.70 - 33.49
4-6 year	Infected	20	36.45 ± 4.39 a	30.35 - 45.67
	Uninfected	10	25.32 ± 2.31 b	22.74 - 28.01

* The different letters refer to the significant differences between the groups.

Table (2): Comparison of serum MCP-1 level in patients infected with *E. histolytica* and in healthy controls

Groups		No. samples	Serum level of MCP-1 (pg/ml)	
			(Mean $\pm$ SE)	Range
1 month- 2 year	Infected	28	28.92 $\pm$ 1.48 a	26.83 - 32.19
	Uninfected	10	18.44 $\pm$ 0.74 c	17.12 - 19.37
2-4 year	Infected	12	30.85 $\pm$ 3.78 a	27.68 - 39.24
	Uninfected	10	22.62 $\pm$ 6.25 b	16.83 - 30.54
4-6 year	Infected	20	31.91 $\pm$ 6.82 a	27.40 - 53.05
	Uninfected	10	18.30 $\pm$ 1.43 c	16.27 - 20.22

* The different letters refer to the significant differences between the groups.

Table (3): Comparison of serum TNF- α level in patients infected with *E. histolytica* and in healthy controls

Groups		No. samples	Serum level of TNF- α (pg/ml)	
			(Mean $\pm$ SE)	Range
1 month- 2 year	Infected	28	58.05 $\pm$ 6.90 a	51.17 - 73.47
	Uninfected	10	42.91 $\pm$ 2.54 b	39.55 - 45.63
2-4 year	Infected	12	55.87 $\pm$ 3.81 a	52.16 - 63.56
	Uninfected	10	43.54 $\pm$ 3.42 b	38.64 - 48.10
4-6 year	Infected	20	57.32 $\pm$ 8.86 a	49.68 - 75.45
	Uninfected	10	42.64 $\pm$ 3.47 b	38.15 - 47.61

* The different letters refer to the significant differences between the groups.

4. Discussion

The results of present study shows that a significant increase in serum level of IL-8, MCP-1 and TNF- α observed during the infection with *E. histolytica*, which reflect a pro-inflammatory role of these cytokines in the infection [13, 14, 15].

Trophozoite attachment to host epithelial and inflammatory cells and to colonic mucins and bacteria is mediated by a lectin specific for galactose/N-acetyl-galactosamine [16, 17, 18]. The contact of trophozoite with target cell via a galactose-inhibitable amoebic adherence protein will induced IL-8 production, therefore coculture of several human epithelial cell lines, nontransformed human fibroblasts and intestinal smooth muscle cells and human liver cells with *E. histolytica* trophozoites resulted in a 5- to 150-fold increase in IL-8 secretion [6]. The secretory products which derived from *E. histolytica* contain large amount of cysteine proteases, and the last one is the important amoebic virulence factors [19, 20]. It is known that tissue-stable mast cells play a role in the mucosal inflammatory response against *E. histolytica*, and these secretory products induce mast cell activation to produce IL-8, which contributes to tissue inflammatory responses through the early period of human amoebiasis [21, 22]. Yu and Chadee [8] shown that the live *E. histolytica* without contact with target cell can increase the secretion and expression of IL-8 from human colonic epithelial cells, and they observed increased of IL-8 mRNA expression from several human colonic cell lines after stimulation by soluble proteins which is secretory components of live trophozoites.

MCP-1 produced by many cell types, including endothelial, fibroblasts, epithelial and smooth muscle. However, monocyte/macrophages are found to be the

major source of MCP-1 [23]. Kammanadiminti *et al.* [4] demonstrate that the soluble proteins (secreted by *E. histolytica*) can induce expression of the chemoattractant MCP-1 in the epithelial cell lines of colon. *E. histolytica* synthesizes its own prostaglandin E (2) (PGE2) via a novel cyclooxygenase-like enzyme [24]. PGE2 is endogenously synthesized and present in secretory soluble components of amoeba; it can induce IL-8 production in colonic epithelial cells, and this cause release of epithelium cytokines/chemokines such as MCP-1 [25]. MCP-1 plays a great role in the recruitment of monocytes and macrophages (from the bloodstream) to inflamed tissue. It has been recorded that the epithelial cells in inflamed mucosa of intestine is the major source of MCP-1 [26]. In other side, Takada *et al.* [27] demonstrated that stable intestinal macrophages can produced MCP-1 even without inflammation.

TNF is one of the cytokines that contributes in systemic inflammation and has important role in acute phase reaction. The major source of TNF is activated macrophages, and it can be synthesized and secreted by other cell types, such as neutrophils, eosinophils, and mast cells [28]. The association of tissue inflammation with TNF secretion from host cells is a documented characteristics of amoebic infection. The inflammation caused by amoeba is amplified as a result of TNF secretion by enterocytes and macrophages [10]. During tissue invaded, the amoeba will be attached with target host cell via the Gal/GalNAc lectin (galactose/N-acetylgalactosamine inhibitable lectin), and this contact lead to cell killing. The Gal/GalNAc lectin is an important protein during amoebiasis, expressed at the cell surface and is the main target cell-binding protein. During tissue invasion, *E. histolytica* will be encounters with immune cells that become activated and secrete

immune compounds called chemokines. In both intestinal and extra-intestinal amoebiasis, the inflammatory response will be increased in host. There is production of various pro-inflammatory chemokines and cytokines, particularly TNF which is secreted by activated macrophages that attracted to the site of infection by other chemokines [29]. In vitro studies shown that TNF- α induces nitric oxide (NO) production from macrophages, and this nitric oxide (NO) is cytotoxic for *E. histolytica*. The production of TNF- α and nitric oxide (NO) can be done from macrophages (after activation by IFN- γ) in response to live amoeba or amoebic proteins [30].

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المستوى المصلي للحركي الخلوي - 8، بروتين جذب الخلايا أحادية النواة -1 وعامل نخر الورم - الفا

في الاطفال المصابين بأميبا الزحار

هدى منير أحمد ، فاطمة شهاب الناصري

قسم علوم الحياة ، كلية العلوم ، جامعة تكريت ، تكريت ، العراق

الملخص

تعد أميبا الزحار من الطفيليات المعوية الابتدائية التي تسبب داء الاميبات الغازي للنسيج. يمكن للطفيلي ان يحفز انتاج الحركات الخلوية (مثل IL-8، TNF- α) والحركات الكيميائية (مثل MCP-1) من الخلايا الظهارية المعوية. أجريت هذه الدراسة للكشف عن المستوى المصلي للحركات الخلوية IL-8، TNF- α و MCP-1 بأستخدام اختبار المناعة المرتبط بالأنزيم في 60 طفل مصاب بداء الاميبات وذلك لبيان علاقة الإصابة مع أنتاج هذه الحركات الخلوية والمقارنة مع الاطفال غير المصابين (n=30). تضمنت الدراسة ثلاثة مجاميع من الفئات العمرية (1 شهر -2 سنة، 4-2 سنة، 6-4 سنة) لكل من الاطفال المرضى والاصحاء. سجل ارتفاع معنوي في مستوى IL-8 (37.22، 37.35 و 36.45 بايكوغرام/ ملليمتر) في الاطفال المصابين بداء الاميبات مقارنة مع المستوى لدى الاطفال غير المصابين (29.95، 26.90 و 25.32 بايكوغرام/ ملليمتر) في كل المجاميع العمرية (1 شهر -2 سنة، 4-2 سنة، 6-4 سنة، على التوالي). كما كان مستوى TNF- α اعلى بشكل معنوي (58.05، 55.87 و 57.32 بايكوغرام/ ملليمتر) في الاطفال المصابين بداء الاميبات مقارنة مع الاطفال غير المصابين (42.91، 43.54 و 42.64 بايكوغرام/ ملليمتر، على التوالي) في جميع الفئات العمرية (على التوالي). وجد ارتفاع ذات دلالة احصائية في مستوى الحركي الكيميائي MCP-1 (28.92، 30.85 و 31.91 بايكوغرام/ ملليمتر) في الاطفال المصابين بداء الاميبات منها في الاطفال غير المصابين (18.44، 22.62 و 18.30 بايكوغرام/ ملليمتر) في جميع الفئات العمرية (على التوالي). هذه النتائج تعكس دور IL-8، TNF- α و MCP-1 خلال الإصابة بأميبا الزحار.