Immunlogical study in human hydatidiosis before and after surgical removal of hydatid cyst

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Abstract

In this study, We aimed to investigate the benefit of monitoring cases with hydatid cyst by means of immune components in patients before and after 15 days after surgery. IL-1β, IL-2, IgG, IgM, IgA, C3 and C4 were evaluated as were the relationship between these parameters with each other. Mean values of C3 and IgG of patients were significantly higher than those of controls before surgery, While mean values of C3, C4 and IgG of patients remain significantly higher than those of controls after 15 days of surgery. There was a significant correlation between serum levels of IL-1β and C3, IL-2 and IgA, C3 and IgA in patient before surgical removal and healthy controls, while there was correlation near to statistical significance between the different immune component studied in patients after 15days of surgical removal.

دراسة مناعية للمرضى المصابين بالاكياس المائية قبل وبعد الأزالة الجراحية للكيس المائية

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الخلاصة

كان الهدف من هذه الدراسة هو التحري عن فائدة رصد المصابين بالأكياس المائية من ناحية المحتوى المناعي لهولاء المرضى قبل وبعد 15 يوم من اجراء العملية الجراحية لأزالة الكيس المائي. تم قياس الأنترلوكين 1 بيتا، الأنترلوكين 2، والكلوبيولينات المناعية من نوع G, M, A والمتممات C3, C4 وكذلك العلاقة بين هذه المتغيرات مع بعضها البعض. قيم الوسط الحسابي للمتمم C3 والكلوبيليولين المناعي نوع G للمرضى كانت بمستوى معنوية اعلى من مجاميع السيطرة بينما بقيت قيم الوسط الحسابي للمتممات C3, C4 والكلوبيولين المناعي نوع G للمرضى بمستوى معنوية اعلى من الأصحاء قبل وبعد 15 يوم من إزالة الكيس المائي بالعملية الجراحية وكان هناك علاقة معنوية بين المستوى المصلي للأنترلوكين1بيتا والمتمم C3, الأنترلوكين 2 والكلوبيولين المناعي نوع A والمتمم C3 مع الكلوبيولين المناعي نوع A في المرضى قبل ازالة الكيس المائي مع الأصحاء، بينما كان علاقة قريبة للمعنوية الأحصائية بين مختلف المكونات المناعية المدروسة في المرضى بعد 15 يوم من ازالة الكيس المائي مع الأصحاء.

Introduction

Echinococcus infections are among the most dangerous helminthic diseases in human (1). Echinococcus organisms, the cause of echinococcosis (hydatid disease), are parasitic helminthes with life cycles involving a carnivorous definitive host (usually dog or fox) and intermediate host (human, ungulate or rodents) (2). Echinococcus granulosus infection in humans triggers a cellular and humeral response commonly characterized by elevated of some serum immunoglobulines (3). Cytokines are important in the regulation of the immune system and are secreted by a variety of cells in response to self and non-self stimuli. Communication within cells, in the same or

distant anatomical sites, occurs via cytokines which determine the quality and intensity of inflammatory and adaptive immune responses (4). Adaptive immunity include Ab and T cell responses generated by lymphocytes (2). It is well known that hydatid disease induces production of antibodies in human with total and specific Immunoglobulin's increased, T cells (T helper cells) produce distinct patterns of cytokines, Th1, Th2 cross regulate one another because their respective cytokines act antagonistically (5). The aim of this study is to determine the levels of the echinococccus antibodies IgG, IgM, IgA and components of complements C3, C4 and serum levels of IL-1, IL-2 before and after surgical treatment and assessing a possible relations of these parameters with each other.

Materials and Methods

Sera were collected from 20 patients before and 15 days after surgery (5 female and 15 male) aged from (16 to 63 years) from Al-Nu'man hospital in Baghdad, with liver and lung hydatid cyst. Patients clinically diagnosed and surgically confirmed in all of the cases, and 20 healthy controls (males and females) aged (20 to 55). The investigation for those patients and control subjects were done in Immunology section at Central Public Health Laboratory. Serum levels of total immunoglobulin's IgG, IgM, IgA and complement factors (C3, C4) were measured by using Radial immnodiffusion (RID) plate (Diagonal) based on the method of (6) and (7). The results were obtained by analyzer in mg/dl. The normal range was IgG (710 to 1520) IgM (40 to 250) IgA (90 to 310), C3 (84 to 193), C4 (20 to 40) Serum IL-1β and IL-2 levels were measured by enzyme amplified sensitivity immunoassay technique (EASIA) kits Biosource/ Europe S.A.-Rue de I'Industry\ Nivelles- Belgium. The tests were performed according to the manufacturer's instructions. All results are expressed as means of standard deviation (mean± SD) comparison of variables was calculated by non parametric Mann-Whitney U test. The level of significance was set at p 0.05 in all analyses. All analyses were performed using the Minitab software.

Results

The study covered 40 subjects attending Al-Nu'man hospital in Baghdad. They were 20 patients with liver and lung hydatid cyst. Sera were collected before and after 15 days of surgical removal of the hydatid cyst. The differences of immunological variables between patients with hydatid cyst and healthy controls are shown in Table(1).

Table (1) Mean, minimum-maximum and median values of serum immunological variables levels in hydatid cyst patients before surgery and healthy controls values are mean ± SD.

Parameter mean ± SD.	Controls(N=20)	Patients before 15 days of surgical removal (N=20) males (15 +ve) females (5 +ve)	
IL-1B (pg/ml)	47.1± 26.17(10.9-98.1,41.8)	71.7 35.9±16.21(11.4-,34.7)	N.S
IL-2(u-ml)	10.9± 8.2(3.9-36.2,8.1)	11.9± 6.4 (3.3 -31.2 ,11.4)	N.S
C3(mg/dl)	95.3± 22.0(63.5- 151.8 , 92.4)	143.6 ± 75.1(39.9-319.8 ,143.4)	*
C4(mg/dl)	26.9±9.9(14.3-51.7,27.7)	36.9±22.9(4.5-77.3 ,33.8)	N.S
IgM(mg/dl)	133.2± 54.0(31.3-233,133.9)	107.7±89.6(3.1-410.3,96.9)	N.S
IgG(mg/dl)	1123± 333(644.5-1811.5,1071.5)	1481±660(219-2849,1426)	*
IgA(mg/dl)	247.5± 1593(53.2-835,232.1)	299.4±164.9(99.7-614.1,232.3)	N.S

***p<0.05 Mann-Whitney U-test.**

Patients before surgical removal of hydatid cyst showed a significant increase of C3 and IgG levels ($p<0.05$), A slight increase of IL-2 and IgA levels comparing with a slight decrease of IL-1β and IgM (N.S). Also there was a high significant increase of C3 levels in hydatid cyst patients after 15 days of surgical removal (0.001), and also there was significant increase in C4 complement and IgG levels ($P<0.01$), while there was slight increase of IL-2 and IgA and slight decrease of IL-1β and IgM serum levels (N.S) (Table 2).

Table (2) Mean, minimum-maximum and median values of serum immunological variable levels in hydatid cyst patients after 15 days surgical removal of hydatid cyst and healthy controls. values are mean ±SD

Immunological variables	Controls (N=20)	Patients after 15 days of surgical removal (N=20) males (15 +ve) females (5 + ve)	
IL-1β (pg/ml)	47.1±26.17(10.9-98.1,41.8)	46.10±24.17(10.2-94.9,47.45)	N.S
IL-2(u-ml)	104±8.2(3.7-36.2,8.1)	12.5±7.3(5.4-35.3,12.1)	N.S
C3(mg/dl)	95.3±22.0(63.5-151.8.92.4)	187.5±92.3(82.3-409.4,161.2)	***
C4(mg/dl)	26.9±9.9(14.3-51.7,27.7)	51.0±24.7(11.1-77.3,50.9)	**
IgM(mg/dl)	133.2±54.0(32.3-235,133.9)	124.6±70.4(26.1-3086,120)	N.S
IgG(mg/dl)	1123.4± 333(644.5-1811.5,1071.5)	1891±836(779-3158,1907)	**
IgA(mg/dl)	2475±1593(53.2-835,232.1)	305.7±206.9(60.5-164.1,237.6)	N.S

*****(p<0.001),**(p<0.01) Mann-Whitney U-test**

A positive correlation between IL-1β and C3, IL-2 and IgA, C3 and IgA levels was found in patients before surgery and healthy control, but correlation between C4 levels and IgG levels, IgM levels and IgG levels also showed a correlation degree near to statistical significance (Table 3).

Table (3) Correlation among disease severities and serum immunological variables valuated by the Pearson correlation p-value before surgery

Immunological variables	Statistical values	C3	C4	IgM	IgG	IgA
IL-1β	r p	0.489 0.029				0.257 0.274
IL-2	r p		0.056 0.815	0.098 0.680	0.956 0.013	0.049 0.446
C3	r p		0..186 0.432	0.582 0.131	0.949 0.015	0.523 0.018
C4	r p			0.635 0.113	0.401 0.081	
IgM	r p				0.422 0.064	
IgG	r p					0.024 0.919

There were a correlation degree near to statistical significance between IL- 1β and C3, IL-2 and C4, C3 and C4, C4 and IgM, IgM and IgA levels as shown in (Table 4).

Table (4) Correlation among disease severities after surgical removal of hydatid cyst and serum immunological variable evaluation by the Pearson correlation P-value

Immunological variables	Statistical values	C3	C4	IgM	IgG	IgA
IL-1β	r p	0.440 0.052	0.386 0.093	0.357 0.122	0.143 0.547	
IL-2	r p				0.081 0.734	0.360 0.119
C3	r p			0.147 0.535	0.224 0.543	
C4	r p		0.433 0.057	0.386 0.093	0.426 0.061	0.066 0.783
IgM	r p				0.196 0.408	0.388 0.091
IgG	r p					0.226 0.338

In healthy controls there were no correlations among the serum levels of IL1β, IL-2, C3, C4, IgG, IgM, IgA. Only a correlation near to statistical significance between IL-1B and C4 levels (Table 5).

Table (5) Correlation between healthy controls and serum immunological variable evaluated by Pearson P-value

Immunological variables	Statistical values	C3	C4	IgM	IgG	IgA
IL-1β	r p	0.255 0.278	0.397 0.083	0.089 0.708		
IL-2	r p		0.031 0.897			0.032 0.892
C3	r p			0.339 0.144		0.082 0.730
C4	r p			0.064 0.788		0.048 0.840
IgM	r p				0.068 0.775	0.264 0.261
IgG	r p					0.209 0.377

Discussion

Parasites have evolved a varaiety of adaptive strategies evading or even exploiting their hosts immune response. Increasing evidence shows that parasites-derived substances play important role in initiating or maintaining the parasites (8). The Echinococcus organisims are very complex multicellular pathogens and they are highly immunogenic, stimulating proinflammatory cellular responses, significant Ab production, and T cell and other cell mediated responses, in their human and intermediate host (2). Both humoral and T-cell mediated responses constituted of T helper cell-1 (Th1) and T helper cell-2 (Th2) type reactions, seem to play important role against infections and are considered to be regulated by cytokines (9, 10). In the immune response to infections, cytokines produced by Th lymphocytes have a role in regulating antibody isotype production (8, 11). Complement system is a critical part of human inflammatory and immune responses to foreign antigens (12). So host complement system interact with the parasite and damage it (13). The results of the present study confirm the previous view and allow some additional details. According to the data we obtained elevated levels of serum complement proteins C3 before surgical removal of hydatid cyst compared to healthy control and C3, C4 were detected in patients after 15 days of surgical removal of hydatid cyst patients compared to healthy controls which may be because complement triggers inflammatory response by the infection followed by complement activation (10) and activation of the complement cascade leads to elevated levels of many potent pro-inflammatory mediators (12) Previous ex-vitro studies have shown an increase in C3 and C4 cystic Echinococcus patients (10, 14).Our data confirmed data previous published of significant increase in IgG levels before and after 15 days of surgical removal compared to healthy individuals and a slight increase of IgA levels (10, 14, 15, 16, 17). A delayed antibody reduction is observed in patients after surgery (16) and it may be maintained in human host for many years after the cyst surgically removed (2). Slight elevation in IL-2 have been detected in hydatid cyst patients before and after 15 days of surgical removal compared to healthy controls due to humoral and cellular response characterized by elevation in some serum antibodies and cytokines (18, 19, 20). A similar elevated level in IL-2 concentration was detected by (14). The proinflamatory cytokine IL-1, IL-1β is reduced in hydatid cyst patients before and after 15 days of surgical removal compared with healthy control this result is similar to (21). That suggested the selective diminution of immune mediators of inflammation may prevent inflammatory pathology by presence of viable *Echinococcus multilocularis* metacestodes that depressed cytokine release. While (22) found no elevation of IL-1β in cystic echinococcus (14). Study showed decrease in IL-1β cytokine level in cystic echinococcus. IL-1 has o pathogenic role in

several diseases and reducing it's production or blocking it's action is an appropriate strategy for treating patients (23). So we found in our study slight increase in IL-2 and decrease in IL-1β, This opposing dynamics of inflammatory cytokines release may prevent overwhelming and pathogenic inflammation and constitute an appropriate response for attraction of effector cells into the periphrastic tissues with the capacity to limit *Echinococcus multilocularis* metacestode proliferation growth and dissemination (21). We found significant correlation levels of total IL-1β and C3 and this result confirm what has been found recently that C3 act also as regulator of synthesis of certain cytokine IL-1β by human peripheral blood mononuclear cells (24). Also a correlation was found between IL-2 and IgA that indicates the clear correlation between cytokine and Ab profiles in chronic stage of human cystic echinococcus (2).The correlation between C3 and IgA before surgical removal may by explained by the fact that Ab activates complement and acts as an opsonin for various pathogens, and that it was reported that antibodies activated complement cascade and immune complex deposition in patients with hydatid cyst (16). In conclusion, our study investigated regulation of specific immune response in cystic echinococcus, and further investigations will be required to detect concentration's of specific immune response on the prognosis until the end of the second year.

References

1. Al-Saimary, I. E.; Al-Shemari, M. N. & Al-Fayadh, M. M. A. (2010). Epidemiological and immunological findings on human hydatidosis. Medical Practice and Review,1(2):26-34.
2. Zhang, W.; Ross, A. G. & McManus, D. P. (2008). Mechanisms of immunity in hydatid disease: implications for vaccine development. The J. of Immunol.,181:6679-6685.
3. Rigano, R.; Profumo, E.; Ioppolo, S.; Notargiacomo, S.; Ortona, E.; Teggi, A. & Siracusano, A. (1995). Immunological markers indicating the effectiveness of pharmacological treatment in human hydatid disease. Clin. Exp. Immunol., 102: 281-285.
4. Baz, A.; Ettlin, G. M. & Dematteis, S. (2006). Complexity and function of cytokineresponses in experimental infection by *Echinococcus granulosus.* Immunobiol., 211: 3-9.
5. Kakkos, S. A.; Mouzaki, A. & Vagianos, C. E. (2001). Modifications of the immune system caused by the cestode. Echinococcus granulosus: A review Annals of Gastroenterol., 14:91-98.
6. Mancini, G.; Carbonara, A. D. & Herremans, J. F. (1965). Immunochemical quantitation of antigens by single radial immunodiffusion. Immunochemistry, 2:235-254.
7. Fahey, J. L. & McKelvey, E. M. (1965). Quantitative determination of serum immunoglobulins in serum-agar plates. J. Immunol., 94:84-90.
8. Rigano, R. A. E.; Bruschi, F.; Carulli, G.; Azzara, A.; Ioppolo, S.; Buttar, B.; Ortona, E.; Margutti, P.; Teggi, A. & Siracusano, A. (2001). Modulation of human immune response by *Echinococcus granulosus* antigen B and its possible role in evading host defenses. Infection and Immun., 69: 288-296.
9. Arai, K. I.; Lee, F.; Miyajima, A.; Miyatake, S.; Arai, N.; Yokota, T. (1990). Cytokines: coordinators of immune and inflammatory responses. Ann. Rev. Biochem., 59: 783-836.
10. Chandrasekhar, S. & Parija, S. C. (2009). Serum antibody and Th2 cytokine profiles in patients with cystic echinococcosis. Indian J. Med. Res., 130: 731-735.

11. Finkelman, F. D.; Holmes, J.; Katona, I. M.; Urban, J. F.; Beckmann, M. P.; Park, L. S.; Schooley, K. A.; Coffman, R. L.; Mosmann, T. R. & Paul, W. E. (1990). Lymphokine control of *in vivo* immunoglobulin isotype selection. Annu. Rev. Immunol., 8:303-333.
12. Mervi Narkio-Makela, M. D.; Anna-Maija, T. & Meri, S. (2000). Complement C3 Cleavage and Cytokines Interleukin-1b and Tumor Necrosis Factor-ain Otitis Media With Effusion. Laryngoscope, 110:1745-1749.
13. Ferreira, A. M.; Irigoin, F.; Breijo, M.; Sim, R. B. & Diaz, A. (2000). How *Echinococcus granulosus* deals with complement. Parasitol. Today, 16: 168-172.
14. Torcal, J.; Navarro-Zorraquino, M.; Lozano, R.; Larrad, L.; Salinas, J. C.; Ferrer, J.; Roman, J. & Pastor, C. (1996). Immune response and *in vivo* production of cytokines in patients with liver hydatidosis. Clin. Exp. Immunol., 106: 317-322.
15. Daeki, A. O.; Craig, P. S. & Shambesh, M. K. (2000). IgG-subclass antibody responses and the natural history of hepatic cystic echinococcosis in asymptomatic patients. Ann. Trop. Med. Parasitol., 94: 319-328.
16. Bulut, V.; Ilhan, F.; Yucel, A. Y.; Onal, S.; Ilhan, Y. & Godekmerdan, A. (2001). Immunological Follow-up of Hydatid Cyst Cases. Mem Inst Oswaldo Cruz, Rio de Janeiro, 96: 669-671.
17. Khabiri, A. R.; Bagheri, F.; Assmar, M. & Siavashi, M. R. (2006). Analysis of specific IgE and IgG subclass antibodies for diagnosis of *Echinococcus granulosus*. Parasite. Immunol., 28: 357-362.
18. Haralabidis, S.; Karagouni, E.; Frydas, S. & Dotsika, E. (1995). Immunoglobulin and cytokine profile in murine secondary hydatidosis. Parasite. Immunol., 17: 625-630.
19. De matties, S.; Rottenberg, M. & Baz, A. (2003). Cytokine response and outcome of infection depends on the infective dose of parasites in experimental infection by Echinococcus granulosus. Parasite. Immunol., 25:189-197.
20. Rigano, R.; Buttari, B.; De Falco, E.; Profumo, E.; Ortona, E.; Margutti, P.; Scotta, C.; Teggi, A. & Siracusano, A. (2004). *Echinococcus granulosus*-specific T-cell lines derived from patients at various clinical stages of cystic echinococcosis. Parasite. Immunol., 26(1): 45-52.
21. Eger, A.; Astrid kirch, B.; Manfras, P.; Kern, H.; Schulz-key. & Soboslay, P. T. (2003). Pro-inflammatory (IL-1β, IL-18) cytokines and IL-8 chemokine release by PBMC in response to *Echinococcus multilocularis* metacestode vesicles. Parasite. Immunol., 25: 103-105.
22. Bayrakter, M. R.; Mehmet, N. & Durmaz, R. (2005). Th1 and Th2 Inducing cytokines in cystic echinococcosis. Turkey Parazitol. Derg., 29 (3): 167-170.
23. Dinarello, C. A. & Wolff, S. M. (1993). The Role of Interleukin-1 in Disease. N. Engl. J. Med., 14 (328):106-113.
24. Takabayashi, T.; Vannier, E.; Clark, B. D.; Margolis, N. H.; Dinarello, C. A.; Burke, J. F. & Gelfand, J. A. (1996). A new biologic role for C3a and C3a des Arg: regulation of TNF alpha and IL-1 beta synthesis. J. Immunol., 156:3455-3460.