Study of some immunological variables resulted from Balb/c mice injection with Hydatid cysts protoscolex antigens

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Abstract

Human cyst disease is a common epidemic disease between humans. The current study was carried out to study some of immunological parameters (the immunoglobins IgG, IgA, the complement proteins C3, C4, the MIF of PMNs cells, the reducing of NBT and the phagocytosis of *Candida albicans*) in the Balb/c mice injected with antigen of protoscolex. By using (15) BALb/c mice and the antibodies IgG, IgA in addition to the complements (C3, C4) that were measured as well as the Migration Inhibition Factor (MIF). The results showed that there were significant differences in IgG (13458) mg/dl. IgA (4773) mg/dl, C3 (359.7) mg/dl, C4 (90.80) mg/dl levels with injection of 0.5 ml in muscle and the MIF levels (1.49) with injection of (0.75) ml. so between the test and the control up ($p \leq 0.05$). This might be a good candidate for immunization and diagnosis of Hydatid cysts in the intermediate host of *E. granulosus.*

Keywords: *Complement, Echinococcus granulosus, Hydatid cyts, Immunoglobulins, Protosocolex antigen*

Introduction

Human cyst disease is a common epidemic disease between humans and animals since ancient times; this disease was caused by the larval stage of granular cestodes of the genus *E. granulosus* [1-5] . The lifecycle of this parasite requires two hosts, the final host, such as dogs, which carry adult worms, and the central host, such as humans, who infected with the larval phase that cause water cyst disease. The disease is endemic in southern America, northern Canada, Western Europe, the Mediterranean basin and the Asia center, and in Australia [6-8] . The Hydatid cysts infect the organs and tissues of the body [9-11] and liver infection represent more than half of the organs' infections[12], While spinal cord and brain injuries are very rare[13], the bone injury was first described by Didlon in 1870. [14] The survival of the parasite in the body may extend for 53 years [15] and this long survival may explain its ability to regulate the immunological response [16] .

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Therefore, current studies have focused on knowing how to stimulate the immune system in the central host and the possibility of using its Antigens of whole-numerical components. Therefore, the present study focused on the possibility of preparing antigens from protoscolex proteins and using different concentrations of these antigens in order to stimulate the immune response in laboratory animals by immunization of the mice with protoscolex antigens using two concentrations intramuscular.

Material and Methods

Experimental design -:

1. The experiment dealt with 15 white mice distributed into three groups of 5 mice each.

2. The first group was injected with a concentration of 0.5 ml of protoscolex antigen I/M. The second group was injected at a concentration of 0.75 ml of the same antigen. The third group injected at a concentration of 0.1 ml PBS solution as a control group at the same location.

3. Measurement the level of serum IgG, IgA in immunized mice using single radial immunodiffusion

assay.

Measurement of neutrophil PMNs and Testing the Phagocytosis of *Candida albicans* by dye exclusion method according to [17].

4. Measurement of NBT dye reductively according to [18].

5. Measurement Coefficient of inhibition of cell migration and Complement measurement using proliferation of a single immune protein in solid pits.

Statistical analysis: mean and standard deviation calculated using SPSS program.

Results and Discussion

The results of the study revealed a significant increase in IgG and IgA antibody production levels in the plentiful serum of mice. The highest concentration of IgG and IgA were recorded respectively (4773) and (3458) mg / 100 ml at antigen concentration 0.5 ml compared to control.

Table (1) Serum levels of IgG and IgA in mice immunized with hydatid cyst protoscolex antigens

Antigen Concentration	Serum levels of IgG and IgA	
	IgG mg/ 100ml	IgA mg/ 100ml
0.5	5 3458. 2 ± 280	4773 ± 47. 9
0.75	3327.8 ± 166.6	452.2 ± 22.8
PBS	1013.3 ± 0.87	1385.2 ± 0.87

Concentration of complement proteins C4, C3 mg/100 ml. in white mice immunized with hydatid cyst protoscolex antigen

Significant increase appeared in the production levels of C3 - C4 passage proteins in the antigen – immunized group using 0.5ml compared to the control group, where the study recorded a consecutive concentration (90.80) and (359.7) mg/100 ml compared to 0.75 ml of antigen ,where the concentration were 70.98 ± 0.36 and 322.8 ± 26.67 respectively as indicated in Table 2.

Table (2) complement proteins concentration in mice immunized with two different dose of hydatid cyst protoscolex antigen

Antigen Concentration	Complement proteins Concentration rate	
	C4 Concentration rate (mg/ 100ml) ±SD	C3 Concentration rate (mg/ 100ml) ±SD
0.5	90, 80 ± 2. 79	359. 7 ± 13. 84
0.75	70. 98 ± 0. 36	322.8 ± 26.67
PBS	55.68 ± 1.13	166.8 ± 16.91

NBT reduction results indicate the presence of significant differences between two different doses of antigens in mice. And it reached the highest 22.74 ± 2.17 in 0.5ml and 0.75 ml of the antigen compared with the control (15.29%). (Table 3)

Table (3) indicates the effect of immunization of white mice with two doses of protoscolex antigen on NBT reductions.

Antigen Concentration	NBT reduction percentage
0.5	22.74 ± 2.17
0.75	23.38 ± 3.73
PBS	15.29 ± 0.83

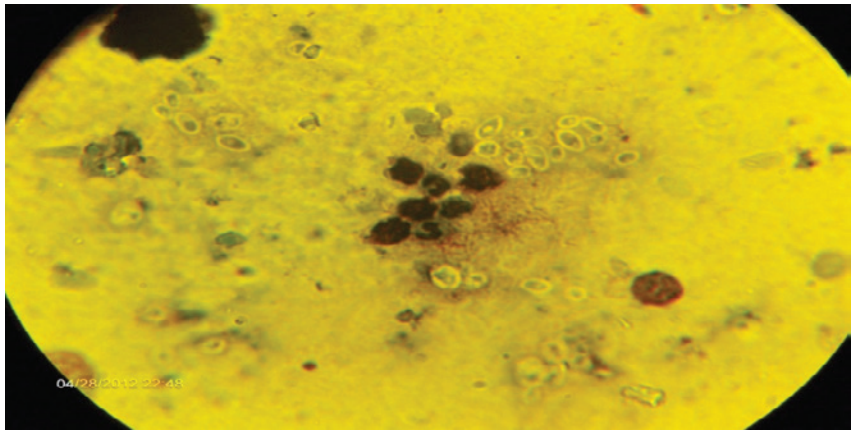

Figure (1) the Reduction of NBT by protoscolex antigen of hydatid cyst.

The results showed significant differences in the Migration Inhibition Factor (MIF) for the two concentrations of the antigen in the muscle compared control as showed in Table (4)

Table (4) MIF test in immunized mice with hydatid cyst antigen under p≤0.05.

Antigen Concentration	MIF rates in mm	MIF
0.5	22.29 ± 0.72	1.47 A
0.75	23.38 ± 3.73	1.49 A
PBS 0.1	15.29 ± 0.83	1 B

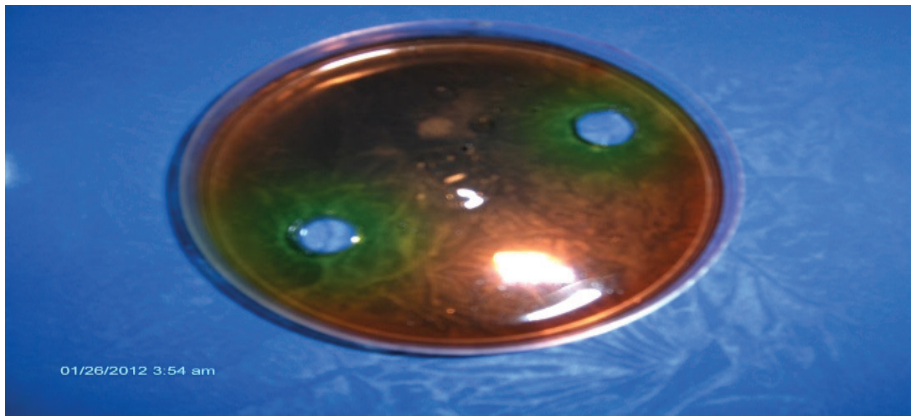

Figure (2) Migration Inhibition Factor under effect of Protoscolexx antigen.

Effect of hydatid cyst protoscolex antigen immunization of white mice with two doses of protoscolex antigen on phagocytosis coefficient of C. albicans.

There were no significant differences between of all groups at all times and the highest percentage was (84.33%) in 30 minutes compared to the control group (80%. 48) as indicated in Table (5).

Table (5) Effect of immunization of white mice with two doses of primary antigenic antigen on *Candida albicans* yeast.

Antigen concentration	30 min	60 min	90 min	120 min
0.5	83.21± 1.41	84.25± 1.41	83.21± 1.54	83.31± 1.22
0.75	84.33± 0.58	81.36± 1.24	83.59± 1.15	70.94± 2.87
PBS	80.48± 0.81	81.44± 1.06	83.25± 1.55	77.08± 0.61

Protoscolex antigens are highly effective in stimulating and activating the immune system, especially in non-lethal doses so that non- expansion of both liver and spleen resulted from the determination of the disease by the immune response of the host, where the spleen is the most resistant organ because it is an immune organ that includes cells that regulate the immune response[18]. The immune response against cystic adenocarcinoma involves the production of fixed levels of antibodies, but these levels are rapidly reduced after removal of the cyst and its organs[20]. IgG levels rise within 2 to 11 weeks of infection[21, 13]. Where the antibodies act in killing the protoscolex of *E. granulosus* worms in the chronic stages of infection, mainly IgG, IgM IgA, antibodies[22- 24]. These levels begin to decline in the chronic stages of the infection [25-27] but start to rise in the later stages[28,29]. Where levels of IgG1, IgG3 significantly increased during the eighth week after the challenge dose and subsequent periods[30]. The immune response mediated by complement and gastric proteins by bodies Antibiotics can be generated by administering two doses of the vaccine within a month and lasting up to 13 months[24]. There is evidences that showed a significant increasing in PMNs and macrophage and eosinophils [31, 32] . some cytokines that are secreted by lymphocytes type - T2 cells can inhibit the effectiveness of the influencing factors despite the stimulation of large numbers of immune cells [33, 34] .

The production of IFN gamma, IFN alpha, interleukins when stimulating cells with antigens plays an important role in the secondary response. Those materials work in inducing T- independent antigens, which in turn stimulate the production of antibodies type IgG3 IgM [22] as there is a clear link between the production of antibodies and the production of cytokines in the chronic stages of infection in humans in IgG4 and IgE types[35, 36]. This binding is necessary to determine the growth of the parasite and prevent the destruction of the organ in the host. The ability of cells to phagocyte germs and antigens depends on their ability to produce super oxide ion, which plays a key role in the mechanism of cell killing, gobbling and destroying antigens when assembling H_2O_2, O_2. [37] However, they do not stimulate lymphocytes qualitatively, but they do not produce sufficient amounts of MIF [13] .

Conclusions

Finding of this research support involvement of immune system competent in mice vaccinate with *E. granulosus* antigens, and in the sequential promotion of the immune cell responses and a modulate immunity in infective animals to moderate vaccines.

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Conflict of Interest- Nil.

Ethical Clearance: Taken from the Scientific Committee College of Veterinary Medicine, University of Fallujah.

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