EVALUATION OF SOME CYTOKINES IN IRAQI PATIENTS BEFORE AND AFTER SURGICAL REMOVAL OF HYDRATED CYST

Waheeda Rashid Ali* and Lamyaa Ali Hussain

Department of Biology, College of Education for Pure Science (Ibn Al-Haitham), University of Baghdad, Iraq.

SUMMARY

Cystic Echinococcosis (CE), also known as Hydatidosis, is one of the most important parasitic diseases in the world. Postsurgical relapses or treatment failure is a frequent risk and a long-term clinical and serological follow-up is required to evaluate the success and failure of therapy. Therefore, the present study was aimed to identify the serum cytokine that correlate with the effectiveness of surgical treatment. The relation of serum cytokine levels was evaluated in 52 patients with cystic Echinococcosis (CE). Serum, IL-6, IL-8, IL-10, IL-12, TGF-β1 and TNF-α concentrations were determined by ELISA before and after surgical treatment.

The result showed the Incidence of CE was higher in females than in males and most infections 33.33% were at the age group (21-30) year. Patients with large medium cyst were more than those with small cyst. The serum cytokine levels of TNF-α, IL-6, IL-10, IL-12 and TGF-β1 were elevated in significant proportion of patients during the active stage of disease in patients and after the surgical treatment showed that, IL-6, IL-10, IL-12, TGF-β1 and TNF-α levels were decreased significantly (P<0.01) however before and post-surgery there was no significant change in IL-8 cytokine levels. Clear from the foregoing that the kinetic cellular IL-8 co-through at least partly in the host defense mechanism against disease and human hydatid cyst.

CONCLUSION

The results indicate that the serum cytokines are useful immunological demonstrating the effectiveness of surgical and pharn logical treatment in the markers follow–up of patients with cystic Echinococcosis.
INTRODUCTION

Cystic Echinococcosis (CE) is a chronic infection caused by small teniid- tapeworm, In humans, CE probably of the estimated 2 to 3 million global cases (Budke et al., 2006.[1] Iraq, hydatidosis it is still a major economic and public health problem, as there is not yet an organized national control program. Disease is endemic and enzootic Iraqi province study of Al-Shammary (2002)[2] recorded the number of people infected E. granulosus in humans triggers a humoral and cellular response characterized by elevated serum antibodies and by concurrent intervention of T helper cell 1(Th1) and T helper cell 2 (Th2) cytokines.[3] Th1 cytokines are related to disease resistance, whereas Th2 cytokines are related to disease susceptibility and chronicity.[3]

The variability and severity of the clinical expression of the disease probably also reflects the variety of human immunological responses to the parasite. Therapy of CE is primarily surgical, even though pharmacological treatment with benzimidazole carbamates is now a days an effective alternative.[4] Clinical evaluation of the outcome of the disease is difficult and relies on combined imaging methods and serological techniques. Because specific antibodies persist in patient's serum for several years after recovery, a long-term clinical and serological follow up is required to evaluate the success or failure of therapy. Most studies on C, E cytokines are mainly based on in vitro experiments, i.e. determination of cytokine production following stimulation of peripheral blood mononuclear cell or T helper cells of patients with crude and B hydatid antigen[5], these studies demonstrated an increase in the production of some cytokines such as IFN-γ, IL-4 and IL-5. Others demonstrated simultaneous involvement of the Th1 and Th2 cells in CE patients.[6] Although two studies have reported the presence of cytokines in sera of surgically treated CE patients, neither of them evaluated the association between cytokines and the clinical outcome of the disease.[7] The aim of this study is to determine serum levels of TNF-α IL-6, IL-8, IL-10, IL-12 and TGF-β1 before and after surgical treatment.

METHODS AND MATERIALS

Blood samples were obtained from 52 patients (9 males and 43 females; range age 5-60 years, with Clinically/Radiologically diagnosed Hydatidosis, tested before and after surgery (one day before and 4-6 day after surgical removal of hydatid cyst) and 50 sex and age-matched healthy controls. They did not present inflammatory disease (not smoker) or any
sign of infection at the time of blood sample collection. Samples from all subjects were centrifuged at 2000xg for 10 minutes to obtain the serum. The lipemic or hemolyzed sera were discarded. The sera was divided in to 3 tubes for each subject and stored immediately at -20°C until cytokine analysis. Clinical diagnosis was surgically confirmed by the presence of cysts in each case (Department of Surgery and laboratory of parasitology, Medical city hospital/Baghdad and Province Bakuba hospital general, from 1-9-2014 to 1-4-2015.

Cytokine detection
Levels of IL-6, IL-8, IL-10, IL-12, TGF-β1 and TNF-α in serum samples were evaluated by enzyme-linked immunosorbent assays (ELISA) according to the manufacturer’s instructions (Pepro Tech, USA). The plates were read on an ELISA reader at 450nm. These assays detected only human cytokines. The samples concentrations calculated by a standard curve fitting equation that performed in the same procedure for each cytokines.

Statistical analysis
Serum level of cytokines was analyzed using the computer programme Statistical Analysis System –SAS, 2012 Their data were given as mean ± standard error (S.E.) and LSD (Least Significant Difference). The percentages have been tested differences between them using the Chi-square.

RESULTS
In the present study, the age of patients varied between 5 and 60 years. The highest incidence of disease was recorded among patients between 16 to 45 years (Table-1). Hydatidosis was found to be predominant in females (82.69%) than in males (17.31%) (Table-2). The study showed that medium-size cyst are the most prevalent, Fifteen patients (68.00%) had cysts 6-10 cm in diameter (Table-3).

Detection of serum cytokines
Serum cytokine levels of IL-6, IL-8, IL-10, IL-12, TGF-β1 and TNF-α were detected in patients’ sera at the time of surgery (pre-surgery) and post-surgery (3-7days). IL-6 levels (Table-4 Fig, 1) were significantly higher (P<0.01) in patients (pre-operative) v/s the healthy controls, the same data clearly shows that post surgery at the indicated time, there was a significant decrease (P<0.001) in IL-6 levels when compared to the pre-operative levels. From this data it becomes obvious that IL-4 can serve as a reliable marker for the success of such surgery, however post-surgery there was no significant change in this cytokine levels.
The IL-10 (Fig, 3) levels were significantly higher (P<0.01) in patients (pre-surgery) v/s the healthy controls, although there was a significant decrease in the post-surgery.

The IL-12 and TGF-β1 levels (Table- 4, Fig 4, 5) were also elevated significantly in patients as compared to healthy controls.

Table -1: Distribution of hydatidosis patients according to age sex.

<table>
<thead>
<tr>
<th>Age(yr.)</th>
<th>Female</th>
<th>Male</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>5-15</td>
<td>3(%6.98)</td>
<td>2(%22.22)</td>
<td>5</td>
</tr>
<tr>
<td>16-30</td>
<td>17(%33.33)</td>
<td>3(%39.53)</td>
<td>20</td>
</tr>
<tr>
<td>31-45</td>
<td>16(%37.21)</td>
<td>4(%44.44)</td>
<td>20</td>
</tr>
<tr>
<td>46-60</td>
<td>7(16.28)</td>
<td>0(0.00)</td>
<td>7</td>
</tr>
<tr>
<td>Total</td>
<td>43(%100)</td>
<td>9(%100)</td>
<td>52</td>
</tr>
</tbody>
</table>

($\chi^2$)Chi-square value ** 10.713 ** 9.835 ----. (P<0.01) **

Table-2: Distribution of hydatid patients and control according to Sex.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Female</th>
<th>Male</th>
<th>Total</th>
<th>Chi-square ($\chi^2$) value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Patients</td>
<td>(% 82.69) 43</td>
<td>(% 17.31) 9</td>
<td>52</td>
<td>12.963**</td>
</tr>
<tr>
<td>Control</td>
<td>(% 80.00) 40</td>
<td>(20.00) 10</td>
<td>50</td>
<td>12.250**</td>
</tr>
</tbody>
</table>

(P<0.01) **

Table -3: Distribution of hydatid patients according to size cysts.

<table>
<thead>
<tr>
<th>Size(cm.)</th>
<th>Small 1-5</th>
<th>Medium 6- 10</th>
<th>Larg11-15</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number</td>
<td>13(17.33%)</td>
<td>51(68.00%)</td>
<td>11(14.6%)</td>
<td>75</td>
</tr>
</tbody>
</table>

($\chi^2$)Chi-square value ** 12.061

**(P<0.01).

Table-4: Serum cytokine levels in healthy control and hydatid patients befor and after treatment.

<table>
<thead>
<tr>
<th>Cytokine</th>
<th>Patients with CE(n=52)</th>
<th>Healthy Control(n=50)</th>
<th>Prey- surgery (rang conc.)</th>
<th>Post- surgery (rang conc.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>IL-6</td>
<td>6.38±0.78</td>
<td>29.16±4.18(0.1-168)</td>
<td>14.67±1.71(0.1-55)</td>
<td>7.49**</td>
</tr>
<tr>
<td>IL-8</td>
<td>0.71±0.05</td>
<td>0.61±0.03 (0.35-1)</td>
<td>0.63±0.03(0.35-0.98)</td>
<td>0.209NS</td>
</tr>
<tr>
<td>IL-10</td>
<td>0.027±0.035</td>
<td>1.49±0.035 (0.06-2.79)</td>
<td>1.23±0.18(0.1-3.16)</td>
<td>0.423**</td>
</tr>
<tr>
<td>TNF</td>
<td>0.002± 0.040</td>
<td>2.01 ± 5.41(0.02-5)</td>
<td>0.15 ±0.462 (4.2-0.04)</td>
<td>3.29 **</td>
</tr>
<tr>
<td>IL-12</td>
<td>6.8±0.82</td>
<td>13.48±0.62(2-100)</td>
<td>17.68±0.80(1-100)</td>
<td>3.778*</td>
</tr>
<tr>
<td>TGF-β1</td>
<td>1.52±0.05</td>
<td>1.31±0.02( 0.9-16)</td>
<td>3.35±0.04(0.1-5)</td>
<td>0.060**</td>
</tr>
</tbody>
</table>

LSD value

** *(P<0.01).
**P<0.05, *P<0.01, Not significant (NS).

**Figure 1** compare between difference group in IL-6.

**Figure (2)** compare between difference group in IL-8.

**Figure (3)** compare between difference group in IL-10.
DISCUSSION

In this study, the sex distribution showed that the predominance of hydatidosis was in females than in males. This has been also observed by \cite{8, 9}, where they found that the infection rate was higher in females than in males. This may be due to the social life in our country.
where the females are confined to house working and this will make them more exposed to the source of infection especially in rural areas. However, this result does not agree with the findings of Frayha et al.,\cite{10} and Al-Najar\cite{11} who observed higher rates of infection in males than in females. From these findings one cannot draw a conclusion on humans infected with hydatidosis. Different authors have reported different results concerning the sex. Therefore, this may be probably due to epidemiological or occupational factors.

The age range of the cases studied was from 5 years up to 60 years. It was shown that the age range between 16-45 years was the age of highest incidence. These findings are consistent with those recorded in the study of Al-Ubaid\cite{12}, which showed an increase in the rate of these age groups. however, the results of this study differed with those obtained by Al-Qadhi\cite{13}, which indicated that the high incidence of the parasite within the age group (11-40 years) may be due reason for the difference in results to the difference in the division of age groups. The cysts of *E. granulosus* never infiltrate or invade the tissues of the host and hence exist as a foreign body. In this study a high percentage of our patients had medium size cysts (6-10 cm in diameter).

One of the most immediate needs in the post-surgical or post-pharmacological treatment immune surveillance of CE patients is to identify markers indicating the effectiveness of treatment. Mezioug and Touil-Boukoffa\cite{14} reported that Th1 response is more related to resistance, protective immunity and killing the cyst, whereas Th2 response is associated with susceptibility to disease and escapes immunity. Zhang and McManus\cite{15} stated that when a cyst dies naturally, is killed by chemotherapy treatment or is removed by surgery, Th2 responses drop rapidly and Th1 responses become dominant. This can be interpreted as Th1 lymphocytes contribute significantly to the inactive stage of hydatid disease, with Th2 lymphocytes being more important in the active and transitional stages.

To our knowledge the current current study is the first study in Iraq to investigate the role of Th1, Th2 and Treg cytokine in CE patients, the cytokine levels of IL-6, IL-8, IL-10, IL-12, TGF-β1 and TNF-α hydatid patients before and after surgery.

The high levels of TNF-α and low levels of IL-6 and IL-10 (Th2) in patients who had responded successfully to treatment are in agreement with many studies.\cite{16,17} Ortona *et. al.*, (3) observed the decrease in IL-4 and IL-10. Increase in TNF-α (Th1) response in patients who responded successfully to treatment, Echinococcus infection, the high level of TNF-α
protein might be associated with the apoptosis of monocytes, which may inhibit the host immunological function.\textsuperscript{18}

The chemokine IL-8 is produced by polymorphonuclear PMN cell\textsuperscript{19} and has been shown to stimulate both neutrophil migration and activation and enhanced antibody-dependent neutrophil cytotoxicity. The current study showed no significant difference in level IL-8 between the study groups, this was consistent with the sort of study Refik et al.,\textsuperscript{20} observed that IL-8 is increased in 11/28(39.3%), while a study by\textsuperscript{21} recorded an increase in the level of IL-8. This difference can be attributed to the location and number of hydatid cyst, patients population and the disease state when blood sample were obtained differed considerably from those in our study, which may explain the differences in the patterns of cytokine response.

The production of IL-12 stimulated by the contact of monocytes and dendritic cells with activated T cells. It stimulated also through the interaction of monocytes, macrophages with extra cellular matrix components that expressed selectively during inflammation.\textsuperscript{22} Serum IL-12 level is significantly higher in patients with hydatidosis than in control subjects. Furthermore, cytokines secretion correlates with disease statues (cystic localizations and clinical stage). Our results provide evidence that level of IL-12 is higher in hydatid patients. This increase is in agreement with previous data on the role of cytokines in host anti-hydatid defence, and underscores the ability of the larval stage of \textit{Echinococcus granulosus} to trigger cytokine production.\textsuperscript{23} The higher serum IL-12 levels observed after surgery support the notion of a relationship between clinical stage and cytokine induction. Our results imply that the increase in cytokines levels correlates with the inflammatory state following surgical removal of cysts. These observations are in line with those reported by many other authors showing that major surgery induces a series of inflammatory responses such as elevation of body temperature and erythrocyte sedimentation rate, leukocytosis and increased acute phase reactants.\textsuperscript{24}

Transforming growth factor-beta (TGF-\(\beta\)) is a multipotential cytokine are induced by different molecules or components of CE, so that AgB could induce TGF-\(\beta\) and components of protoscolex, other than AgB and Ag5, could induce IL-10.\textsuperscript{24} TGF-\(\beta\) plays an important role in Alveolar Echinococcus both in immune tolerance against the parasite and in liver fibrosis.\textsuperscript{25} little is known about TGF-\(\beta\) involvement in the pathophysiology of larval Echinococcosis. Only preliminary studies are available in \textit{Echinococcus}: Zhang et al.\textsuperscript{26} showed that TGF-\(\beta\) was expressed in most lymphocytes of the periparasitic infiltrate in liver
biopsies from *Echinococcus* patients we found that TGF-β increased significantly in patients after surgery while it decreased in untreated patients. For untreated patients, their decreased concentration may due to the role of TGF-β in disease onset[27] and in the clearance of inflammation by apoptosis induction in T cells.

**REFERENC**


