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LONG-TERM EVALUATION OF PATIENTS WITH HYDATIDOSIS TREATED WITH ALBENDAZOLE AND PRAZIQUANTEL

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Hydatidosis is a usually asymptomatic chronic disease. In most patients who undergo surgery, hydatidosis is not resolved due to high recurrence rate. However, long-term treatment with albendazole has been found to have a significant efficacy that has been further improved when albendazole is combined with praziquantel and fat-rich diet. In this study a retrospective evaluation of the outcome of hydatidosis in 70 patients, was performed. In group A, a combined chemotherapy of albendazole plus praziquantel was given after surgical removal of cysts. In group B chemotherapy alone was administered without surgery. Sera of all patients were assayed for IgG, IgM, IgA and IgE antibodies by ELISA. In addition, ultrasonography (US) and/or computerized tomography (CT) scans were performed every 3 months for 18 months, and then, each year until the end of follow-up. The difference between the two kinds of treatment used in the present study was found to be not significant, nor was the difference of the shrinkage and extended calcification of the HCs between the two groups. However, the difference of the shrinkage of the HCs of more than 80%, as well as the extended calcifications of the cysts between the two groups were found to be statistically significant. In all patients high levels of IgG and IgA were detected, while IgE in group A and/or IgM in group B were marginally detected above the background level throughout the study. Level of IgG was strongly fluctuated and significantly decreased at 11.7 years after the end of chemotherapy, or at 8.5 years after relapses in group A, while was dramatically decreased at 3.6 years after the termination of chemotherapy in group B. Relapses occurred in 11.4% of patients within the first six months after end of chemotherapy. After additional chemotherapy with albendazole for 3-6 months, all of them were considered cured at 8.5 years of follow up.

Hydatidosis is a chronic, spontaneously curable disease, which can rarely threaten health. However, it is likely to create some serious health problems, after medical management of the hydatid cyst (HC), if biology of the parasite is not essentially taken into consideration. Surgical intervention of HC is usually applied for surgically accessible cysts in life-threatening locations or often for cysts which are

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compliant to surgery (1).

In humans, HC is usually an "accidental finding" during x-ray examination (2). Although most patients undergo surgery, the problem cannot be solved because of the high recurrence rates and the high risk for the life of patients (3). In Greece, surgery is usually recommended as primary treatment of hydatidosis with a morbidity and mortality during the last 20 years of 18% and 3%, respectively (4).

Administration of benzimidazoles, isoquinolones, etc., once engaged for inoperable cases of hydatidosis, is very effective against the parasite in humans (3, 5-7, 9). After nearly 23 years of use, albendazole is the main medication in the management of hydatidosis, if the liver is able to derive albendazole sulphoxide, the drug's principal metabolite (5). Long-term albendazole treatment has significant efficacy in about 70% of the cases (11) and is considerably improved when albendazole is combined with praziquantel and food (7, 12). A better interpretation of the situation during follow-up gives the combination of radiology with serology, because cysts suffer relatively small changes that radiology cannot appreciate (2), and the immunodiagnostic techniques can be used for diagnosis as well to follow-up of patients after surgical or pharmacological treatment, or both (13).

In this study we address questions concerning diagnosis and treatment of hydatidosis. For this purpose we studied 70 hydatidosis patients clinically, serologically and with imaging methods for 13.5 years. Patients were retrospectively divided in two groups. In the first group patients were given post-operative albendazole plus praziquantel, while in the second group patients received chemotherapy alone (no surgery).

MATERIALS AND METHODS

Patients

Seventy patients (42 female and 28 male) diagnosed with hydatidosis were retrospectively divided into two groups. Group A consisted of patients who underwent chemotherapy post surgery and group B consisted of patients who underwent chemotherapy without surgical treatment.

Chemotherapy

All patients were treated with: i. albendazole, 10 mg/kg per day in cycles of 30 days, followed by intervals of 15 days without treatment (5). Daily dosage was divided into three portions given every 8 hours after meals, for better absorption of the drug, and ii. praziquantel, 50 mg/kg per day for seven continuous days and then, 50 mg/kg per week for two months (7).

History and symptoms, as well as adverse effects during chemotherapy, were recorded. Apart from detection of specific antibodies, all patients underwent clinical, blood count and chemistry examination, including liver enzymes, every month (at the intervals of chemotherapy) and US or CT assessment every 6 months. Chemotherapy outcome was evaluated by either reduction in size or disappearance or calcification of the cyst, and remarkable reduction or disappearance of specific antibodies. Chemotherapy stopped once the level of specific IgG had notably declined, HC was essentially calcified (inside or/and cyst wall), compared with previous scan, and specific IgM, IgE and IgA were undetected. The study lasted for 13.5 years. After completion of chemotherapy, the final evaluation of degeneration of HCs performed at the end of follow-up ranged from 3.6 years (group B) to 11.7 years (group A).

Sera

Serum samples were collected from all patients in the Hospitals of Thessaloniki (424 A.G.T.H., Gennimatas Hospital, Hippokration Hospital and A.H.E.P.A. Hospital) before chemotherapy, every month for 19 months and every year until the end of follow-up 13.5 years after chemotherapy (January 1993 until June 2006). The sera were sent to the Department of Parasitology and Parasitic Diseases, Veterinary Faculty, Aristotle University of Thessaloniki, where they were kept at 4°C and were tested 2-3 days later. Specific IgG antibodies were estimated in the sera on the above-mentioned dates. The level of specific IgM, IgA and IgE antibodies were approximated before chemotherapy, every 3 months for 18 months and then, every year until the end of follow-up, for 3.6 years in group B to 11.7 years in group A of patients.

Antigen

Sheep hydatid fluid and brood capsules were obtained from liver cysts. Brood capsules containing protoscoleces were collected by centrifugation at 1500 rpm for 10 min at 4°C and washed three times with 0.01 M PBS (phosphate buffered saline, pH 7.2). About 1000 protoscoleces were sonicated at 22 kHz for 2 min at 4°C, centrifuged at 3700 rpm for 10 min at 4°C and the protein content (20 mg/ml) was determined in the supernatant, according to the Biuret method. The supernatant was stored at –20°C for subsequent use as antigen in ELISA.

Serological test

Sera were diluted 1:300 for IgG, IgM, IgA and IgE

Table I. *Group and age of patients, organ and number of hydatid cysts (HC).*

Organ	Number of patients <17 years	Median value (SD)*	Range*	HC**	Number of patients >17 years	Median value (SD)*	Range*	HC**
Group A of patients								
Liver	3	10 (4)	6-14	3	35	43 (14.55)	18-76	45
Lung	6	10 (1.36)	7-11	6	1	-	-	1
Spleen	-	-	-	-	1	-	-	2
Brain	1	-	-	1	-	-	-	-
Total A	**10**			**10**	**37**			**48**
Group B of patients								
Liver	2	10.5 (3.53)	8-13	2	18	51 (14.42)	18-78	18
Brain	3	2 (4.04)	2-9	3	-	-	-	-
Total B	**5**			**5**	**18**			**18**
Total (A+B)	**15**			**15**	**55**			**66**

** referring to the age of patients*
*** number of hydatid cysts*

Table II. *Duration of chemotherapy.*

Treatment (group)	**Number of patients**	**Months of treatment** (median value, sd, range)	**Cycles of 45 days** (mean)
After surgery (group A)	47	4.5-27 (15, 6.25, 22.5)	3-18 (10)
Chemotherapy alone (group B)	23	4.5-24 (9, 4.67, 22.5)	3-16 (7)

Table III. *Degeneration of HCs in the original 70 patients after chemotherapy at the end of follow up.*

Degeneration of HC	**Number of patients (%)**	**Group (patients)**	Statistical differences between A & B group
Moderate calcification	52 (74.3)	Group A (44) and group B (8)	p 1.000
Shrinkage 20-80% and extended calcification	14 (20)	Group A (3) and group B (11)	p <0.0005
Shrinkage >80% and extended calcification	3 (4.3)	Group B (3)	-
Disappearance	1 (1.4)	Group B (1)	-

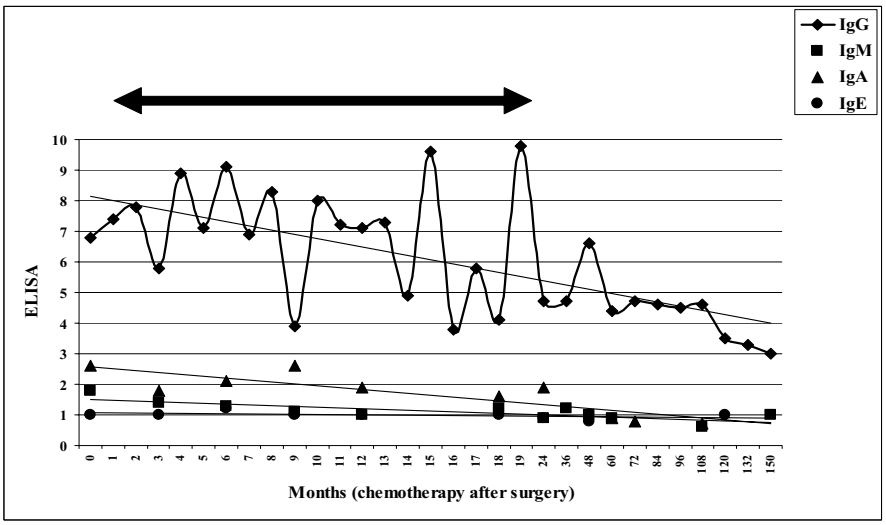

Fig. 1. *Serum levels of specific immunoglobulins before, during and after chemotherapy in 38 patients (group A) suffering from liver hydatidosis and undergoing chemotherapy after surgery (double arrow = maximum duration of chemotherapy).*

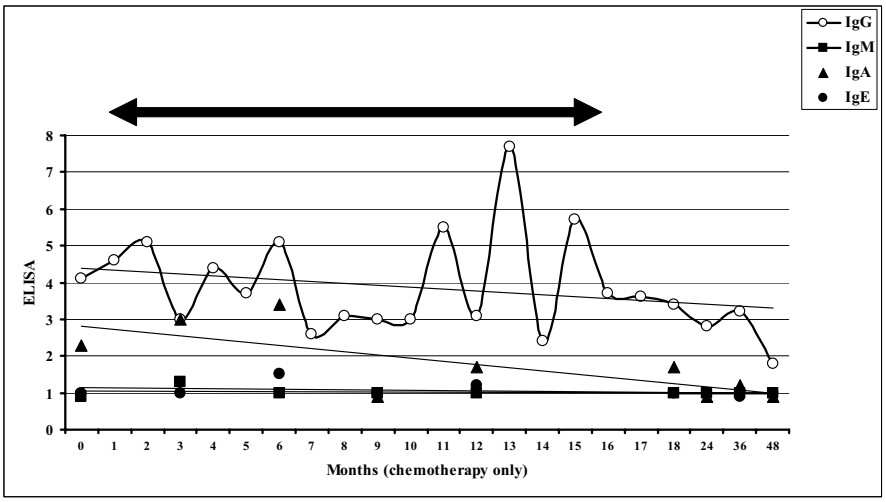

Fig. 2. *Serum levels of specific immunoglobulins before, during and after chemotherapy in 20 patients (group B) diagnosed with liver hydatidosis and treated with chemotherapy alone (double arrow = maximum duration of chemotherapy).*

detection and were tested by enzyme linked immunosorbent assay (ELISA) using 10 μg of antigen/ml (8).

Statistical analysis

Data were analyzed by using the two sample z tests (significance level was set to α=0.05) and the chi-square test.

RESULTS

In group A (47 patients) 58 HCs were found. In vital organs (Table I), i. forty-eight HCs were found in the liver of 38 patients aged under 17 years (3 patients, median value 10, sd 4, range 6-14 years, 3 HCs) and over 17 years (35 patients, median value 43, sd 14.55, range 18-76 years, 45 HCs), ii. seven HCs in the lungs of 7 patients aged under 17 years (6 patients, median value 10, sd 1.36, range 7-11 years) and over 17 years (one patient 70 years old), iii. two HCs in the spleen of a 54-year old patient, and iv. one HC in the brain of a 5-year old patient.

Similarly, in group B twenty-three HCs were found in the liver and the brain of 23 patients (Table I). Twenty HCs were found in the liver of 20 patients aged under 17 years (2 patients, median value 10.5, sd 3.53, range 8-13 years) and over 17 years (18 patients, median value 51, sd 14.42, range 18-78 years), and 3 HCs in the brain of 3 patients aged 2-9 years (median value 2, sd 4.04, range 2-9 years).

In total, 81 HCs were found in the organs of the 47 patients of group A (58 HCs) and of the 23 patients of group B (23 HCs, Table I). In group A, 33 patients had one liver cyst each, three patients had 3 liver cysts each, one patient had 4 liver cysts, one patient had 2 liver cysts, seven patients had 1 lung cyst, one patient had 2 spleen cysts and one patient had 1 brain cyst. In group B, 20 patients had 1 liver cyst each and three patients had 1 brain cyst each.

The diameter of the 68 HCs established were 6±3.8 cm in liver, 7±2.5 cm in lungs (7 cysts), 12.5±0.7 cm in spleen (2 cysts) and 3±1.4 cm in brain (4 cysts).

Eighteen of the patients in group A complained of abdominal discomfort involving either the liver (17 patients) or the spleen (1 patient). No patients of group B referred any symptom.

Four more patients were not included in our study because of pregnancy, liver cirrhosis, complete calcification of cyst or advanced age (>90 years old).

Chemotherapy

Duration of chemotherapy ranged: a) either 4.5 to 27 months (median value 15 months, sd 6.25, range 22.5 months) or 3 to 18 cycles of 45 days each (mean 10 cycles) in group A, and b) either 4.5 to 24 months (median value 9 months, sd 4.67, range 22.5 months) or 3 to 16 cycles (mean 7 cycles) of 45 days each in group B (Table II).

The overall cure rate at the end of chemotherapy was 88.6% (41 patients/87.2% of the 47 patients in group A and 21 patients/91.3% of the 23 patients in group B, p 0.297), and increased either to 100% of the 70 patients at the end of the 13.5 years of evaluation or 3.6 (group B) to 11.7 years (group A) after the end of chemotherapy.

At the end of follow up, moderate calcifications, inside or/and in the cyst wall, were observed in 52 (74.3%) patients (44/group A and 8/group B, p 1.000) of the original 70 patients (Table III). Extended calcifications and shrinkage 20-80% (appearance of cyst as a solid calcified mass) were observed in fourteen (20%) patients (3/group A and 11/group B, $p < 0.0005$). In three patients (4.3%) in group B, liver cysts were reduced in size by more than 80%, and in one other patient (1.4%) in group B, a liver cyst sized 5 cm disappeared (Table III). In total, extended calcifications or 80-90% shrinkage or disappearance of cysts was observed in 18 patients (25%) of the 70 patients. Of these patients, 15 were in group B (65.2% of 23 patients) and 3 in group A (6.4% of 47 patients, $p < 0.0005$).

High levels of specific IgG and IgA were detected during chemotherapy in group A (Fig. 1) and in group B (Fig. 2), while low levels of specific IgM were only detected during the first 6 months of treatment in group A (Fig. 1). Specific IgE in group A (Fig. 1) or/and IgM in group B (Fig. 2) were marginally detected above the background level throughout the study.

The level of IgG in both groups fluctuated strongly during chemotherapy, with peaks frequently observed after the end of each cycle of treatment.

In group A (Fig. 1), IgG level was significantly decreased at 11.7 years after the end of chemotherapy, or at 8.5 years after relapses, while IgG in group B (Fig. 2) was dramatically decreased at 3.6 years after the end of chemotherapy.

After the end of chemotherapy, high levels of specific IgG were detected for 7 months in 7 patients (group A) with cysts in lungs and for 2 months in 3 patients (group B) with cysts in the brain.

Relapses occurred in 8 patients (11.4%), within the first six months after the end of chemotherapy, so that specific IgG, IgM and IgA were elevated during the first 2-3 months after the end of chemotherapy. Of these patients, five in group A and two in group B had one liver cyst each. An additional patient in group A had one cyst in the lungs. All of them responded favourably to a further 3-6 months chemotherapy with albendazole, and cysts were considered cured at 8.5 years of follow up. There was no difference in the relapse rate between the two groups.

Adverse effects were observed in six (8.6%) patients of group A, described as: i) mild elevations of ALT and AST during the first 1-2 cycles of treatment in three liver and one pulmonary hydatidosis cases (all measurements reversed to the normal levels on cessation of chemotherapy); ii) mild

pain and malaise in the entire body of one patient after a daily treatment with a dose of albendazole 3-fold higher than normal, for 12 days (symptoms disappeared in a few days when patients returned to the regular dose of regime); and iii) severe alopecia in a child with pulmonary hydatidosis, that started as heavy loss of hair and developed to complete loss of hair two to three weeks after daily treatment of a 10-fold higher dose of albendazole than normal. Hair grew back again one month after treatment with a regular dose of albendazole. All adverse effects gradually resolved after cessation of chemotherapy for one month after which chemotherapy continued without any adverse effects. No adverse effects were observed in group B.

No association was found between response to treatment and age of patient, organ and size of cyst.

DISCUSSION

Pre- and post-operative chemotherapy is used as rule to prevent relapses, as well as to decrease the danger of spillage during surgical intervention (14-15). From the parasitology point of view, protoscoleces are resistant to all anti-parasitic drugs used against them during intervention or for short term treatments. These "incarcerated" parasites that are physiologically destroyed 5 to 20 years after the infection or some months after medication (3, 6), are regularly released during surgical treatment and cause the production of new cysts (relapse). Surgical treatment seems to be an "unhopeful gift" for the parasite, whilst for the patient it represents a "scientific error" (this is not the case for cysts found in a location threatening the human life). According to our study, efficacy of chemotherapy in patients with no surgical intervention for removal of HC proved more advantageous than to those who underwent pre- or/and post operative chemotherapy. Furthermore, our results are similar to rates achieved by Chai et al (16) using albendazole alone, which showed cure rates of 60.6-75.1% at the end of treatment, and 83.9-84.2%, 2-4 years post-treatment.

The effectiveness of albendazole ranged between 70% and 99.1%, depending on factors such as location and size of HC, follow-up duration etc. (9, 11, 16-18). According to Todorov et al (18) the first degenerative changes occurred 1-3 months (small cysts <5 cm) or 2-5 months (large cysts) after initiation of treatment, and then cysts became smaller, and finally disappeared 3.3-9.3 months (small cysts) or 5.6-13.9 months (large cysts) after treatment began. The overall rate of decrease or disappearance of the cysts was 25% in our study, which is similar to percentages observed by Saimot (19), but lower than 39% and 57% observed by Todorov et al (20) and Nahmias et al (9), respectively. Our rate of 65.2% for group B coincides with the results found by Nahmias et al (9), but not for group A, which was 6.4% ($p < 0.0005$). A possible reason for these big differences between the two groups of patients could be the insufficient blood circulation around the cyst, because the connective tissue, enhanced locally after surgical intervention of HC (group A) in a way that allowed entry of medication inside the cyst, are impeded. The continuous entry and the presence of albendazole sulphoxide inside HC deter the growth of new parasites and the size of the cyst, while it accelerates the physiological calcification of the cyst.

Specific antibody detection is a valuable method of monitoring patients after treatment. The predictable value of ELISA in follow-up studies of patients with hydatidosis (2, 21-23), and the well-known sensitivity of the assay that is not influenced by either size or type of cyst (2) ensures the objectivity of the results.

Taking the previously published findings and the results of our study together, we conclude that chemotherapy should be used as first-line treatment in hydatidosis, yielding more complete decrease or disappearance of the HC when surgery has not been performed. Moreover, the serum levels of specific antibodies as influenced by the status of the hydatid cyst can be used as a guide to evaluate the outcome of chemotherapy and the clinical management of hydatidosis. Therefore, the close cooperation among clinicians and parasitologists is considered necessary.

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