

Some Immunological Aspects in Sera of Iraqi Patients Infected with Hydatid Cysts

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ABSTRACT

A disease causes hydatid cysts called cystic echinococcosis (CE), one of the most important zoonotic diseases due to the infection with *Echinococcus granulosus* tapeworm. Bio immunological markers play an important role in human immunological response during infection with hydatid disease. This study aimed to determine levels of some chemokines such as (IP-10, MIP-1 α , MCP-1) and CA19-9 tumor markers in 105 subjects 50 of them were CE patients compared with 25 healthy individuals and 30 cancer patients. All bio immuno-markers in sera were carried out using enzyme-linked immunoassay (ELISA) commercial kits. Interferon gamma-induced protein 10 (IP-10) was elevated in all CE patients (106.566 ± 10.422 pg/mL). Macrophage inflammatory protein (MIP-1 α) concentrations were higher in the sera of the CE group (143.026 ± 15.842 pg/mL). Levels of Monocyte chemoattractant protein-1 (MCP-1) in serum are significantly higher in CE patients (202.546 ± 40.406 pg/mL). All data were compared with the values obtained from healthy individuals and showed statistically significant differences at ($p < 0.01$) in all chemokine levels. A statistically significant correlation between CA19-9 and hydatid disease and cancer was found. CA19-9 levels were elevated in the CE and cancer patients groups which recorded (52.071 ± 11.796 pg/mL) and (96.102 ± 13.262 pg/mL), respectively, significant differences from the healthy control group (5.401 ± 2.446 pg/mL).

Keywords: CA19-9, Cystic echinococcosis, Hydatid cysts, IP-10, MCP-1, MIP-1 α , Immuno-markers.

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INTRODUCTION

Hydatid cysts disease or CE is a very important parasitic zoonoses disease and widespread caused by tapeworm (Cestoda), family Taeniidae and the genus *Echinococcus*.¹ Adult stages of this parasite live in the intestine of the carnivores, which consider definitive hosts such as dogs, hyenas, foxes, lions, and leopards, then transmitted to the herbivores as an intermediate host which includes cows, goats, buffaloes, sheep, camels, pigs, rabbits, donkeys, horses, and humans, where the hydatid cysts larvae live.²

Echinococcus granulosus and *E. multilocularis* are two main species had medical and public health importance. The hydatid cysts of *E. granulosus* develop in the intermediate host's internal organs, including humans, as fluid-filled bladders.³ These cysts can be located in the liver and lungs at ratios of 70 and 20% respectively, as well as in other organs such as the brain, spleen, kidney, bone, and heart.⁴ Furthermore, the hydatid cysts are slow-growing and able to survive for several years in chronic infections. On the other hand, humans become infected with this parasite by ingesting food or water contaminated with the parasite's eggs or by direct contact with animal hosts or contaminated soils.^{5,6}

The clinical expressions of the parasitic diseases reflect the variety of human immunological responses to the parasite.⁷ The immune response has a great role in the host-parasite interaction in cystic echinococcosis. Through the early stages of *Echinococcus* worm infection, there is a noticeable activation of cell-mediated immunity, cellular inflammatory responses, and pathological changes.⁸ One of the characteristics of *E. granulosus* and *E. multilocularis* infection is the production of Th₁ polarized cytokine in the early stages could be able to kill the metacestode.⁹ then a response shifts to Th₂ as a predominant cytokine in the chronic stage. Th₂ response is associated with susceptibility to the disease, whereas a Th₁ response is related to protective immunity.¹⁰

Previous methods of diagnosing infections with hydatid cyst disease in humans using ELISA,¹¹⁻¹³ or radiological techniques.^{14,15} This study focused on determining the infection by hydatid cysts in the patient's sera of liver hydatidosis at three types of chemokines (IP-10, MIP-1 α , and MCP-1) when compared to healthy people. In addition, to measure tumor marker levels CA19-9 in sera of liver hydatid cysts patients and liver cancer patients, to evaluate a relationship between this biomarker and other related components of the immune

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response in patients of hydatid cysts disease and healthy people.

MATERIALS AND METHODS

Samples Collection

In the current study, a total of 105 venous blood samples were collected from Iraqi recumbent and incoming patients to the department of surgery of Al-Imamain Al- Kadimain medical city in Baghdad from March to August 2021. The samples were divided into three groups; the first group included 25 (control) people whose clinical diagnosis showed no one had any inflammatory infection at the time samples were collected. The second group included 50 patients with hydatid cyst infections who were ready for eradication surgery in this hospital. The Clinical and radiological diagnoses were made on the patients using ultrasonography before. The third group included 30 patients with symptoms similar to the second group and diagnosis of cancer. The age of individual participants ranged from 33 to 60 years, and verbal consent was taken from all individual participants for inclusion in this study.

Diagnosis of Immunological Chemokine’s Levels

Five ml of venous blood for each sample (control group and both patients’ study groups) were drawn and separated by centrifugation at 3000 g for 5 minutes to get the serum, then was coded with numbers and stored at -20°C until used.

Each serum sample was determined for immunological Humans chemokine’s levels in it and included levels of IP-10, MIP-1α, and MCP-1. ELISA commercial reader was dependent on measuring levels of (IP-10, MIP-1α, and MCP-1) using their kit which is supplied by (Peprotech, USA) and CA19-9 (Human, Germany). The manufacturer’s instructions were followed in each of these kits. The results were expressed in pg/mL.

Statistical Analysis

The data results were analyzed statically by measuring the mean with standard deviation. Data analyses were performed with the statistical package for the social sciences using (SPSS, version 14). The minimum significance level was taken at the *p*-value < 0.05.

RESULTS

Determination levels of IP-10

Analysis of circulating chemokines productions reveals that IP-10 levels were elevated significantly at (*p* < 0.01) in CE patients (106.566 ± 10.422 pg/ mL) compared to the healthy control group, which is calculated (65.41 ± 11.038 pg/mL) as shown in Table 1.

Determination levels of MIP-1α

The levels of MIP-1α was elevated in hydrated cyst patients and determined (143.026 ± 15.842 pg/mL) significant at (*p* < 0.01) in CE patients, as shown in Table 2 compared with the uninfected control cases were determined (61.123 ± 13.131 pg/mL), and there were significant differences between the two studied groups.

Determination levels of MCP-1

Elevated levels of MCP-1 were determined in CE patients as (202.546 ± 40.406 pg/ ml) compared to the control group, which was registered (80.745 ± 10.460 pg/ mL) and there were significant differences between patients and the healthy control group at (*p* < 0.01). The results of these levels are summarized in Table 3.

Figure 1 shows the consolidated result of three chemokines levels of IP-10, MIP-1α and MCP-1 were detected in this study compared with their controls. Figure 1 columns show that the

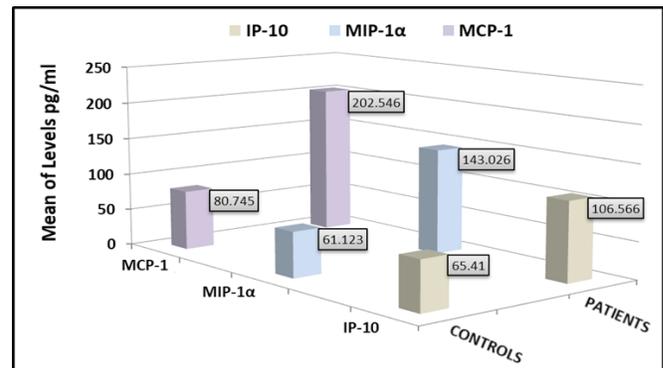


Figure 1: Chemokine’s levels of IP-10, MIP-1α, and MCP-1 in pg/mL compared with their controls at *p* < 0.01

Table 1: Levels of IP-10 in CE and control groups estimated in pg /mL.

STAT		N	Min.	Max.	Mean ± Std. deviation	CS
IP-10	Control	25	44.50	85.40	65.41 ± 11.038	<i>p</i> ≤ 0.01
	Patient	50	80.80	122.20	106.566 ± 10.422	

Table 2: Levels of MIP-1α in CE and control groups estimated in pg /mL.

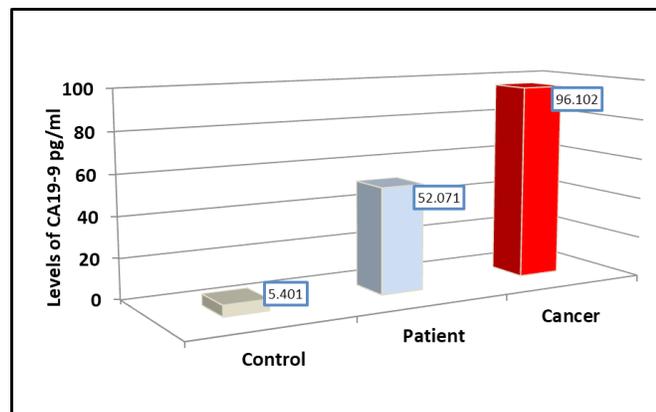
STAT		N	Min.	Max.	Mean ± Std. deviation	C.S
MIP-1α	Control	25	34.8	95.6	61.123 ± 13.131	<i>p</i> ≤ 0.01
	Patient	50	111.2	170.2	143.026 ± 15.842	

Table 3: Levels of MCP-1 in CE and control groups estimated in pg /ml.

STAT		N	Min.	Max.	Mean ± Std. deviation	CS
MCP-1	Control	25	55.50	95.30	80.745 ± 10.460	<i>p</i> ≤ 0.01
	Patient	50	116.1	280.50	202.546 ± 40.406	

Table 4: Levels of CA19-9 in CE, control, and cancer groups estimated in pg/mL.

STAT		N	Min.	Max.	Mean \pm Std. deviation	CS
CA19-9	Control	25	1.5	10.1	5.401 \pm 2.446	$p \leq 0.01$
	Patient	50	25.5	70.5	52.071 \pm 11.796	$p \leq 0.01$
	Cancer	30	66.88	123.6	96.102 \pm 13.262	$p \leq 0.01$

**Figure 2:** Levels of CA19-9 pg/ml in the serum of patients who have cancer compared with CE patients and control at $p < 0.01$

levels of MCP-1 were higher than two others were registered at 202.546 pg/mL compared with MIP-1 α and IP-10, which it's detected as 143.026 pg/ mL and 106.566 pg/mL, respectively.

Determination levels of CA19-9

The levels of a tumor marker CA19-9 showed a significant increase ($p < 0.05$) in serum among patients with CE (Table 4) were calculated as (52.071 \pm 11.796 pg/ mL) compared with the control group (5.401 \pm 2.446 pg/mL). The results show a high increase in the level of CA19-9 tumor marker in the cancer patients group was determined (96.102 \pm 13.262 pg/mL) with a significant difference compared with the levels of patients and control groups that are shown in Table 4 and Figure 2.

DISCUSSION

Parasitic infection is suggested to be one of the significant causes of morbidity and mortality in developing countries.¹⁶ Cystic echinococcosis is one of the common infections in Iraq and is considered a chronic disease. In humans as intermediate hosts, CE cysts can parasitize for a long time, and it forms a complicated microenvironment in the peripheral immune system.¹⁷

Despite the development of modern imaging techniques and treatment strategies, the problems related to the diagnosis and treatment of patient's cases with CE still require solutions.¹⁸ A rapid and early diagnosis is very important to control diseases and leads to reduced morbidity and mortality, so the current research aims.

Three chemokines have been selected to apply in this study. Chemokine IP-10, MIP-1 α , and MCP-1 in serum levels were screened in CE patients and the controls.

The current result of IP-10 showed high levels in CE-infected patients (106.566 \pm 10.422) compared to the control group (65.41 \pm 11.038). The levels of IP-10 are

significantly higher in CE patients compared to normal healthy individuals. Moreover, these data demonstrated that IP-10 might be a valuable biomarker for detecting the disease in the chosen sample. Also, IP-10 was selected for diagnosing different parasitic diseases.^{19,20,21} Similar results were observed for this research.

The levels of MIP-1 α chemokine were found to be significantly increased ($p < 0.001$) in patients with hydatid cyst (143.026 \pm 15.842) while the control subjects were (61.123 \pm 13.131). CCL2/MCP-1 is a chemokine secreted by different cells in their response to inflammation.²² The levels of MCP-1 were elevated in CE patients (202.546 \pm 80.745) and showed a significant increase compared with the control group (80.745 \pm 10.460).²³ Moreover, the result data demonstrated that the Chemokines IP-10, MIP-1 α , and MCP-1 could be valuable biomarkers for detecting and diagnosing the disease in any chosen sample.²⁴ In clinical oncology, the rapid expansion of detection and identification techniques for applying new tumor markers has highly increased and become a trending technology because it gives an understanding of the human body and its diseases.²⁵

Previously published studies demonstrated that the CA19-9 tumor marker is associated with some cancer cases. Where the elevated levels of CA 19-9 may indicate cancer and noncancerous conditions.²⁶ In this study, CA19-9 was applied to detect hydatid cysts and cases of cancer patients. The levels of CA19-9 in the patients' serum of CE cases (52.071 \pm 11.796), compared with the control group (5.401 \pm 2.446). While the levels of CA19-9 in the cancer patients group were so high (96.102 \pm 13.262), as well as showed significant differences between cancer patients compared with the CE patients and control groups. These results agree with the findings,²⁷ where CA19-9 levels were significantly higher in CE-infected patients in the presurgical compared with the controls.

CONCLUSION

The findings indicate an elevated increase in levels of three chemokines, particularly chemokine of IP-10, MIP-1 α , and MCP-1 in serum levels screened in CE patients compared with those in the controls. Furthermore, a determination of tumor marker of CD19-9 in the samples study showed a high response in cancer cases with significant values than results of CE patients. Finally, more studies must be conducted using a combination of cyst-associated immune parameters that can be applied to detect and diagnose hydatid cyst disease.

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