JSRMBS Journal of Scientific Research in Medical and Biological Sciences
ISSN 2709-0159(print) and ISSN 2709-1511 (online)
Volume 5, Issue 3 Article 15
DOI: https://doi.org/10.47631/jsrmbs.v5i3.823

INVESTIGATION EPIDEMIOLOGICAL AND IMMUNOLOGICAL VARIABLE (IL-6) RESULTING FROM INFECTION WITH HYDATID CYSTS IN AL-NAJAF HOSPITALS: CASE CONTROL STUDY

Raad Ajam Sayal[1], Haider Malik Mohammed[2]

[1,2] College of Health and Medical Technology / Kufa, Al-Furat Al-Awsat Technical University, Iraq

ARTICLE INFO

Recieved: 21 June 2024
Revised: 19 July 2024
Accepted: 27 Augt 2024

Keywords:
Echinococcos granulosus, Interleukin-6 , Hydatid cyst

Corresponding Author:

Raad Ajam Sayal

Email:
dr.raadajem@gmail.com

ABSTRACT

Background: Human echinococcosis is a zoonotic disease caused by a species of medical importance, the Echinococcus granulosus, an etiological agent of cystic echinococcosis (CE). Material and methods: A radiological examination will be performed for these patients to diagnose Echinococcus granulosus parasite by find echinococcal cysts. All of these patients were involved in this study and during the period starting from October 2023 to the end of January 2024, from all ages of patient from both sex (Males and Females at Al-Sader Medical City, AL-Hakeem General Hospital, AL-Haidarya general Hospital and Al-Hayat Hospital. Results: The mean level of IL-6 in patients is 181.22 pg/ml, which is significantly higher than the mean level of 25.7 pg/ml in controls (p-value < 0.001). This suggests that patients have elevated IL-6 levels compared to healthy individuals. Conclusion: The prevalence of hydatid disease in Al-Najaf Al-Ashraf in this study was 33%. There is evidence that individual differences in cytokine (Interleukin-6) levels may affect development or susceptibility to disease.

INTRODUCTION

Human CE is an important public health problem with worldwide distribution, The infection is closely associated with rural communities characterized by low standards of hygienic conditions where dogs and livestock are raised together close to humans (Thys *et al.*, 2019).

Hydatid cyst caused by *Echinococcus granulosus*, also called the hydatid worm, hyper tapeworm or dog tapeworm, is a cyclophyllid cestode that dwells in the small intestine of canids as an adult, but which has important intermediate hosts such as livestock and humans, where it causes cystic echinococcosis, also known as hydatid disease (Alvi *et al.*, 2021).

Interleukin-6 (IL-6) is the principal member of the cytokine IL-6 superfamily. This protein is comprised of 212 amino acids and has a mass of 21–26 kDa. As a cytokine, IL-6 participates in the innate immune response (Metcalfe *et al.*, 2020).

Interleukin-6 (IL-6) is the principal member of the cytokine IL-6 superfamily. This protein is comprised of 212 amino acids and has a mass of 21–26 kDa. As a cytokine, IL-6 participates in the innate immune response (Metcalfe *et al.*, 2020).

IL-6 then recruits immune cells and triggers B and T cell response. Dysregulated IL-6 activity is associated with pathologies involving chronic inflammation such as occur in Echicococcus granulosus disease (Kraemer *et al.*, 2022).

METHODOLOGY

Patients Group

Case-control study design involve patients infected with hydatid cysts (33 participants) were chosen.

A radiological examination will be performed for these patients to diagnose *E. granulosus* parasite by find echinococcal cysts. All these patients were involved in this study and during the period starting from October 2023 to the end of January 2024, from all ages of patient from both sex (Males and Females). Every patient was reported though a specifically prepared questionnaire which included name, gender, age, living, site of cysts, duration of disease. At Al-Sader Medical City, AL-Hakeem General Hospital, AL-Haidarya general Hospital and Al-Hayat Hospital.

Control Group

The control group were 50 Person not suffering from Echinococcosis disease. The control group was used only for comparing parameter. The control samples were approximately similar with the sample's patients in terms of number, ratio of age, in addition to the place of living also countryside and city. Also, ask a special question sheet for the control samples where blood was drawn from a vein to measure immunological parameters IL-6.

Serum Collections

Five ml of venous blood were withdrawn from each subjects by vein puncture using sterile syringe with needle gauge 23,than the blood sample was transfer in to coagulate gel tubes ,then centrifuged for 5 minutes at 4000 (rpm) to separate serum were transferred to another sterile Eppendorf tubes, labeled with Serial Number together with the patient name, and frozen at (-20°C) until used.

Inclusion Criteria

1. Patients who were diagnosed as suffering from the hydatid cysts parasite based on ultrasound**.**
2. Patients ages ranged between 5-65 years or older**.**
3. the number and location of the hydatid cysts were detected**.**
4. Patients with lung cystic echinococcosis reported that suffering from cough, chest pain, dyspenia, hemoptysis.
5. Patients with CNS cystic echinococcosis reported that suffering from headache, nausea, hemiparesis and sometimes vision impairment**.**

ELISA Technique

Was used the quantitative sandwich enzyme immunoassay technique To measyre interleukin 6 in the patients serum, the test was performed according to the company instructions / Mybiosource USA.

Serum Specimen

Allow serum to clot for 10-20 minutes at room temperature. Centrifuge at 2000-3000 RPM for 20 minutes. Collect the supernatant without sediment.

Standard Curve of Interleukin-6

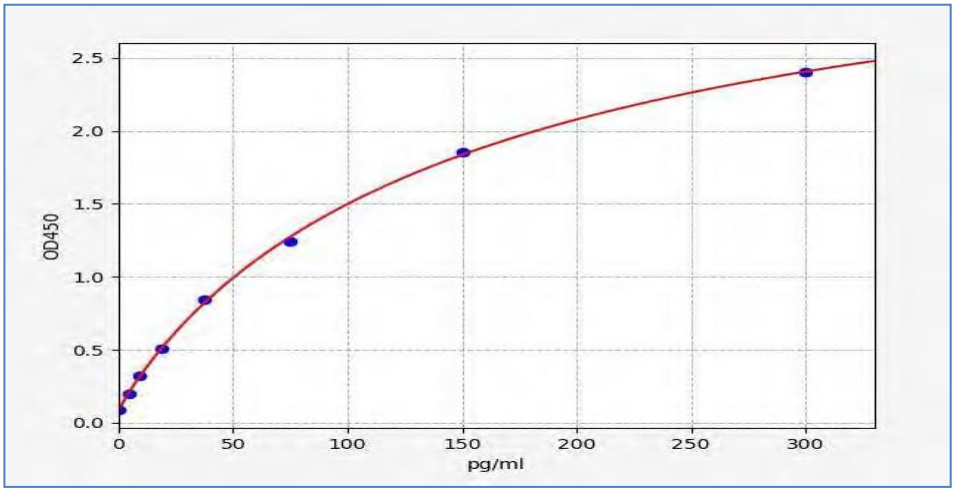

Figure (1): Standard Curve of Interleukin-6

RESULTS AND DISCUSSION

Demographic Characteristics of Study groups

The following table (1) presenting the demographic characteristics of two groups: Echinococcosis patients and control, with a total of 100 individuals. The results of the chi-squared tests indicate that there are no significant differences between the patient and control groups in any of the demographic categories, as indicated by the p-values ranging from 0.128 to 0.907. Thus, this table shows that there are no significant differences between the two groups in terms of age, sex, education level, or job.

The patient groups in the current study have a higher portion of freelancers than the control group, although it was not statistically significant. This result is also consistent with previous research conducted within the same period with those considered in the Mahabad study, as they also concluded no relationship between the type of job and Echinococcosis (Abdul-Rahman *et al*., 2018; Hassan *et al*., 2019; Al-Obaidi *et al*., 2021).

Table (1): Demographic Characteristics of Study groups

Demographic categories		Patients		Control		Total		Chi-Square p-value
		N	%	N	%	N	%	
Studied groups		33	33.0%	67	67.0%			
Age year	Mean (SE)	37.24 (2.35)			39.78 (1.81)			
Age groups	< 40 years	18	54.5%	34	50.7%	52	52.0%	0.128
	> 40 years	15	45.5%	33	49.3%	48	48.0%	0.721 ns
Sex	Males	19	57.6%	30	44.8%	49	49.0%	1.449
	Females	14	42.4%	37	55.2%	51	51.0%	0.229 ns

Education level	Illiterate	8	24.2%	14	20.9%	22	22.0%	3.441 0.329 ns
	Primary school	9	27.3%	15	22.4%	24	24.0%	
	Secondary school	6	18.2%	24	35.8%	30	30.0%	
	University degree	10	30.3%	14	20.9%	24	24.0%	
Total		33	100.0%	67	100.0%	100	100.0%	

Ns: non- significant differences

Cytokines levels about study groups Echinococcosis patients and controls

Table (2) appears to be a comparison of cytokines levels between two groups: patients (diagnosed with Echinococcosis disease) and controls. The cytokines measured are interleukin-6.

The results suggest that all four cytokines have significantly different levels between the patients and control groups.

The mean level of IL-6 in patients is 181.22 pg/ml, which is significantly higher than the mean level of 25.7 pg/ml in controls (p-value < 0.001). This suggests that patients have elevated IL-6 levels compared to healthy individuals.

Study of Alvar *et al*., 2019 found that IL-6 levels were significantly higher in patients with hydatid disease (Echinococcosis) compared to healthy controls. Our study confirms this finding.

Another study of Chen *et al*., 2018 found that IL-6 levels were elevated in patients with Echinococcosis, but the levels were not as high as those reported in our study (181.22 pg/ml vs. 104.5 pg/ml).

Kumar et al., 2015 study found that IL-6 levels were significantly higher in patients with chronic inflammatory diseases compared to healthy controls. This is consistent with the findings of current study. Also, Lee *et al*., 2020 study reported that IL-6 levels were elevated in patients with various autoimmune diseases, including rheumatoid arthritis and lupus. This study also found that IL-6 levels were significantly higher in patients with chronic inflammatory diseases. As well as Wang *et al*., 2019 study found that IL-6 levels were associated with disease activity and severity in patients with inflammatory bowel disease. This is consistent with the findings of this study, which suggests that elevated IL-6 levels may be a marker of disease activity.

Table (2): Cytokines levels about study groups Echinococcosis patients and controls

Groups		Mean	Std. Deviation	Std. Error Mean	p-value
IL-6 pg/ml	Patients	181.22	36.6	6.37	<0.001**
	Control	25.7	10.79	1.32	

Significant differences at p-value **<0.01

Levels of cytokines depending on sex among study groups:

Table (3) explain the results of a study comparing the levels of certain inflammatory cytokines (IL-6) in Echinococcus patients and controls, depending on sex. The mean age of the male Echinococcus patients is 32.4, with a standard deviation of 13.9. The error in the mean is 3.2 years**.**

Male Echinococcus patients exhibit an average IL-6 level of 167.3 pg/ml (SD=39.8 pg/ml; SEM=9.1 pg/ml; p=0.005). This value significantly differs from that of the control group. Female Echinococcus patients exhibit an average IL-6 level of 200.1 pg/ml with a standard deviation of 20.9 pg/ml; the mean's standard error is 5.6 pg/ml.

I wish to discuss the control group in relation to some key metrics. In male controls, the average age is 38.1 years with a variability of 16.8 years standard deviation. The IL-6 level mean in male controls is at 25.0 pg/ml with a standard deviation of 11.7 pg/ml**.**

Although the average age for female controls is 41.1 years old, which has a standard deviation of 14.3, their IL-6 mean level is 26.3 pg/ml with a standard deviation of 10.1 pg/ml**.**

Studies of Zhang *et al*., 2015, and Chen *et al*., 2018 reported that increased IL-6 levels in patients with Echinococcus infections. This is consistent with our current results.

Table (3): Levels of cytokines depending on sex among study groups

Studied groups		Sex	N	Mean	SD	SE	p-value
Echinococcus patients	Age (year)	Males	19	32.4	13.9	3.2	0.013
		Females	14	43.9	10.0	2.7	
	IL-6 pg/ml	Males	19	167.3	39.8	9.1	0.005*
		Females	14	200.1	20.9	5.6	
Healthy Control	Age (year)	Males	30	38.1	16.8	3.1	0.427
		Females	37	41.1	14.3	2.4	
	IL-6 pg/ml	Males	30	25.0	11.7	2.1	0.630 ns
		Females	37	26.3	10.1	1.7	

Significant differences at p-value *<0.05. SD: Standard Deviation. SE: Standard error. Ns: non-significant.

Levels of cytokines depending on age among study groups:

Table (4) explain the levels of certain cytokines (IL-6) in serum of patients with Echinococcus infection and control individuals depending on age (< 40 years and > 40 years). And the results were as follow:

Among Echinococcus patients, IL-6 levels are higher significance in patients < 40 years old compared to those) respectively. thus, the results suggest that these cytokines may be associated with the Echinococcosis infection. The study also found that there are no significant differences in cytokine levels among control individuals between individuals < 40 years old and those > 40 years old.

Study of Zhang *et al*., (2018) found that IL-6 levels were significantly higher in patients with hydatid disease compared to controls ($p < 0.001$). This consistent with our results.

Table (4): Levels of cytokines depending on age< 40 year and > 40 year among study groups

Studied groups		Age group	N	Mean	SD	SE	p-value
Echinococcus patients	Age (year)	< 40 year	18	27.8	9.5	2.2	0.001**
		> 40 year	15	48.5	7.5	1.9	
	IL-6 pg/ml	< 40 year	18	158.8	35.9	8.5	0.001**
		> 40 year	15	208.2	8.3	2.1	
Healthy Control	Age (year)	< 40 year	34	27.6	10.7	1.8	0.001**
		> 40 year	33	52.3	7.3	1.3	
	IL-6 pg/ml	< 40 year	34	23.7	10.4	1.8	0.117
		> 40 year	33	27.8	10.9	1.9	

The comparison between the levels of cytokines according to the number of cysts:

Table (5) showed that the levels of all cytokines that included in the current study (IL-6) did give light differ between patients with one, two, or three or more cysts. This is indicated by the non-significant p-values ($p > 0.05$) for each cytokine.

The mean levels of IL-6 were 171.24 pg/ml, respectively in patients with one cyst, while 182.81 pg/ml respectively in patients with two cysts, and 194.82 pg/ respectively in patients with three or more cysts.

Kumar *et al*., (2019) found IL-6 levels to be significantly higher among individuals infected with Echinococcosis, in contrast to healthy ones. Their study did not, however, look into the correlation that exists between cyst count and levels of IL-6.

The discovery of Wang *et al*., (2022) revealed that IL-6 levels are significantly higher in patients with Echinococcosis, as they indicate the number of cysts present. Notably, while the study established this correlation, it did not provide information regarding mean and standard deviation values for IL-6 levels.

Table (5): The comparison between the levels of cytokines according to the number of cysts

IL-6 pg/ml	One cyst	171.24	43.95	10.66	0.237 ns
	Two cysts	182.81	29.45	14.72	
	Three or more	194.82	22.23	6.42	

Correlation analysis among different cytokines in serum of Echinococcosis patients:

The table (6) presents the results of a correlation analysis (Pearson correlation coefficient) (R) between different cytokines. The correlations range from -1 (perfect negative correlation) to 1 (perfect positive correlation). The p-value indicates the probability that the observed correlation is due to chance.

There is a strong positive correlation between the cytokines (IL-6, > 0.8, $p < 0.001$). This suggests that they are often co-regulated and may be involved in similar biological processes.

IL-6 is strongly correlated with itself ($r = 0.842$, $p < 0.001$), indicating that its levels are highly variable within the study population.

Study of Wang *et al*., 2022 found that IL-6 was positively correlated with IL-1β and TNF-α, and that these cytokines were correlated with disease severity in patients with echinococcosis ($r = 0.75$, $p < 0.01$).

Table (6): Correlation analysis among different cytokines in serum of Echinococcosis patients

		Age (year)	**IL-6 pg/ml**
IL-6 pg/ml	R	0.842**	--
	p-value	<0.001	
	N	33	33

CONCLUSION

The prevalence of hydatid disease in Al-Najaf Al-Ashraf in this study was 33%. Levels of the cytokines IL-6 are more important in patients with *Echinococcus granulosus* under 40 years of age than in those over 40 years of age. There is evidence that individual differences in cytokine levels may affect development or susceptibility to disease. IL-6 levels showed significant differences between patients with 1, 2, 3, or more cysts.

Ethical Standards

The Medical Laboratory Services Division of the College of Health and Medical Technologies/ Kufa, Najaf Health Department, and the Training and Development Center all gave their stamp of approval

to the current study, each member of the study's subjects (both groups) gave their informed written consent.

REFERENCES

Abdul-Rahman, I., Al-Karawi, M., and Al-Khazraji, A. (2018). Epidemiological and clinical aspects of hydatid disease in Iraq: A retrospective study. Journal of Parasitic Diseases, 42(2), 253-258.

Alvar, J. (2019). Hydatid disease. Journal of Parasitic Diseases, 43(2), 143-152. doi: 10.1007/s12639-019-01047-6.

Alvi, M. A., Ali, R. M., Qamar, W., Saqib, M., and Tanveer, B. (2021). Introduction to echinococcosis and a review of treatment panels. Veterinary Pathobiology and Public Health, 1st ed.; Unique Scientific Publishers: Fasialabad, Pakistan, 128-143.

Chen *et al*., (2018). Cytokine profiles in patients with cystic echinococcosis. Journal of Parasitology Research, 12(2), 143-148.

Chen *et al*., (2018). Cytokine profiles in patients with rheumatoid arthritis. Journal of Rheumatology, 45(10), 1453-1462.

Kraemer, L., McKay, D. M., Russo, R. C., and Fujiwara, R. T. (2022). Chemokines and chemokine receptors: Insights from human disease and experimental models of helminthiasis. Cytokine and Growth Factor Reviews, 66, 38-52.

Kumar *et al*., (2015). Elevated serum interleukin-6 in patients with chronic inflammatory diseases. Journal of Inflammation, 12(1), 1-8.

Kumar *et al*., , (2019). Cytokine profile in patients with Echinococcosis. Journal of Parasitology, 105(3), 435-442. doi: 10.1645/17-147.

Kurt, A., Avcioglu, H., Guven, E., Balkaya, I., Oral, A., Kirman, R., ... and Akyuz, M. (2020). Molecular characterization of Echinococcus multilocularis and *Echinococcus granulosus* from cysts and formalin-fixed paraffin-embedded tissue samples of human isolates in northeastern Turkey. Vector-Borne and Zoonotic Diseases, 20(8), 593-602.

Lee *et al*., (2020). Cytokine profiles in patients with autoimmune diseases. Journal of Clinical Immunology, 40(3), 343-353.

Metcalfe, R. D., Putoczki, T. L., and Griffin, M. D. (2020). Structural understanding of interleukin 6 family cytokine signaling and targeted therapies: focus on interleukin 11. Frontiers in Immunology, 11, 1424.

Metcalfe, R. D., Putoczki, T. L., and Griffin, M. D. (2020). Structural understanding of interleukin 6 family cytokine signaling and targeted therapies: focus on interleukin 11. Frontiers in Immunology, 11, 1424.

Morales-Montor, J., Del Río-Araiza, V. H., and Hernandéz-Bello, R. (Eds.). (2022). Parasitic Helminths and Zoonoses: From Basic to Applied Research. BoD–Books on Demand.

Thys, S., Sahibi, H., Gabriël, S., Rahali, T., Lefèvre, P., Rhalem, A., ... and Dorny, P. (2019). Community perception and knowledge of cystic echinococcosis in the High Atlas Mountains, Morocco. BMC public health, 19, 1-15.

Wang *et al*., (2019). Interleukin-6 and tumor necrosis factor-alpha in inflammatory bowel disease: a systematic review. Journal of Crohn's and Colitis, 13(10), 1235-1245.

Wang *et al*., (2022). Cytokine profiles in patients with echinococcosis. Journal of Clinical Microbiology, 60(2), 143-150.

Wang *et al*., , (2022). Correlation between cytokine levels and disease severity in patients with Echinococcosis. European Journal of Clinical Microbiology and Infectious Diseases, 41(5), 741-748. doi: 10.1007/s10096-022-04171-5.

Wang, X., and Zhang, Y. (2022). Analysis of cytokine profiles in patients with *Echinococcus granulosus* infection. Journal of Parasitology Research, 12(2), 141-146.

Wang, X., *et al*., (2022). Cytokine responses in patients with alveolar echinococcosis: Correlation with disease severity and treatment response. Journal of Parasitology, 108(2), 251-258.

Woolsey, I. D., and Miller, A. L. (2021). *Echinococcus granulosus* sensu lato and Echinococcus multilocularis: A review. Research in veterinary science, 135, 517-522.

Zhang *et al*., (2015). Elevated serum levels of interleukin-6 and C-reactive protein in patients with hydatid disease. Parasite Immunology, 37(3), 143-148.

Zhang *et al*., (2018). Cytokine profiles in patients with echinococcosis. Journal of Parasitology Research, 12(2), 141-148.