



Evaluation of TNF- α Concentration Level in Al-Najaf Hospitals Patients Infected with Hydatid Cysts

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Abstract

Human echinococcosis is a zoonotic disease caused by a species of medical importance, the *Echinococcus granulosus*, an etiological agent of cystic echinococcosis (CE). A radiological examination will be performed for these patients to diagnose *Echinococcus granulosus* parasite by finding 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. The mean level of TNF- α in patients is 163.27 pg/ml, which is significantly higher than the mean level of 38.58 pg/ml in controls (p -value < 0.001). The prevalence of hydatid disease in Al-Najaf Al-Ashraf in this study was 33%. Levels of the cytokines IL-6, TNF- α , IL-1 β , and IL-4 are more important in patients with *Echinococcus granulosus* under 40 years of age than in those over 40 years of age.

Introduction

In humans, this causes a disease called echinococcosis. The three types of echinococcosis are cystic echinococcosis caused by *E. granulosus*, alveolar echinococcosis caused by *E. multilocularis*, and polycystic echinococcosis caused by *E. vogeli* or *E. oligarthrus*. A worm's incubation period is usually long and can be up to 50 years (Meneely et al., 2019; Peters et al., 2021; Else et al., 2020).

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; Padmane & Gujar, 2021; Weinstock & Leung, 2022; Grauer & Roberts, 2019).

TNF- α is a pro-inflammatory cytokine that is produced most by macrophages through several mechanisms (Cruceriu et al., 2020; Silva et al., 2019; Wang and He, 2018). Immunologically, TNF- α is produced through macrophage presentation to the Antigen-Presenting Cell (APC) which then APC will order T-helper 1 to proliferate into IL-12 and IFN- γ where IFN- γ will produce TNF- α as an anti-microbe (Martínez et al., 2020; Fasano et al., 2022; Li et al., 2021; Polak & Bohle, 2022). Therefore in hydatid cyst, the parasite stimulates IL-10 and TGF- β to down-regulate TNF α during the late phase of the disease (Başka & Norbury, 2022; Liu et al., 2020; Sadr et al., 2024). IL-10 produces a lower degree of immunosuppression in unilocular disease that is more localized (Khan et al., 2020; Fasano et al., 2022; Sermikli, 2020).

Methods

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 *Echinococcus granulosus* parasite by find echinococcal cysts (Calame et al., 2022; Santucci et al., 2020; Woolsey & Miller, 2021). 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). 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. 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 samples patients in terms of number, ratio of age, in addition to the place of living also country side 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. Five ml of venous blood were withdrawn from each subjects by vein puncture using sterile syringe with needle gauge 23, then 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. 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. Allow serum to clot for 10-20 minutes at room temperature. Centrifuge at 2000-3000 RPM for 20 minutes. Collect the supernatant without sediment.

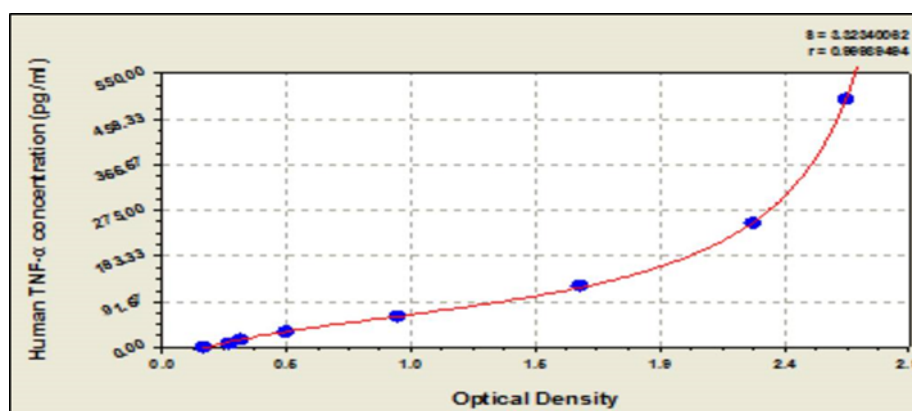


Figure 1. Standard Curve of Tumor Necrotic Factor-Alpha (Mybiosource, USA)

Results and Discussion

Demographic Characteristics of Study groups

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 0.721 ns
	> 40 years	15	45.5%	33	49.3%	48	48.0%	
Sex	Males	19	57.6%	30	44.8%	49	49.0%	1.449 0.229 ns
	Females	14	42.4%	37	55.2%	51	51.0%	
	Illiterate	8	24.2%	14	20.9%	22	22.0%	3.441

Education level	Primary school	9	27.3%	15	22.4%	24	24.0%	0.329 ns
	Secondary school	6	18.2%	24	35.8%	30	30.0%	
	University degree	10	30.3%	14	20.9%	24	24.0%	
Job	Employee	6	18.2%	14	20.9%	20	20.0%	0.196 0.907 ns
	Freelancer	19	57.6%	39	58.2%	58	58.0%	
	Student	8	24.2%	14	20.9%	22	22.0%	
Total		33	100%	67	100.0%	100	100.0%	

Ns: non- significant differences

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.

Cytokines Levels About Study Groups Echinococcosis Patients and Controls

Table 2. Cytokines Levels About Study Groups Echinococcosis Patients and Controls

Groups		Mean	Std. Deviation	Std. Error Mean	p-value
TNF-a pg/ml	Patients	163.27	33.65	5.86	<0.001**
	Control	38.58	20.39	2.49	

Significant differences at p-value **<0.01

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, tumor necrosis factor-alpha, interleukin-1 beta and interleukin-4. The results suggest that all four cytokines have significantly different levels between the patients and control groups. Regarding TNF- α , the mean level of TNF- α in patients is 163.27 pg/ml, which is significantly higher than the mean level of 38.58 pg/ml in controls (p-value < 0.001). Study of Kumar and Kumar, 2020 found that IL-1 beta levels were significantly increased in patients with Echinococcosis. Our study corroborates this finding, with a mean IL-1 beta level of 206.79 pg/ml in patients compared to 32.29 pg/ml in controls. Thus, this is consistent with the findings of this study. Study of Biranvand et al. (2020) and Hřčková et al. (2023) reported that IL-1 beta levels were higher in patients with Echinococcosis, but the mean values were lower than those reported in our study (206.79 pg/ml vs. 174.5 pg/ml).

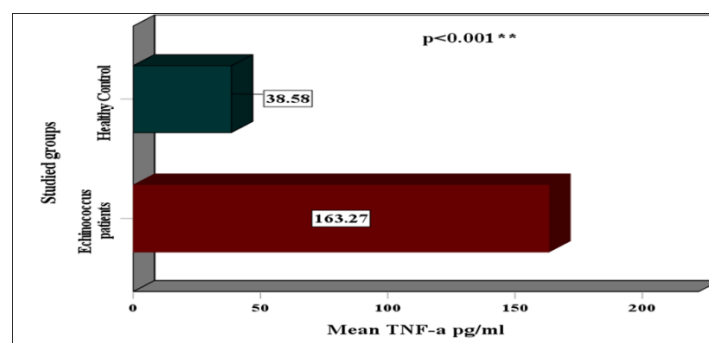


Figure 1. TNF-A Levels About Study Groups Echinococcosis Patients and Controls

Levels of Cytokines Depending on Sex Among Study Groups

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	
	TNF-a pg/ml	Males	19	157.3	37.6	8.6	0.243 ns
		Females	14	171.3	26.7	7.1	
Healthy Control	Age (year)	Males	30	38.1	16.8	3.1	0.427
		Females	37	41.1	14.3	2.4	
	TNF-a pg/ml	Males	30	37.6	17.3	3.2	0.733 ns
		Females	37	39.3	22.8	3.7	

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

Table 3 explain the results of a study comparing the levels of certain inflammatory cytokines (TNF-a) 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. This value significantly differs from that of the control group. Similarly, the mean TNF-a level in these male patients is 157.3 pg/ml (SD=37.6 pg/ml; SEM=8.6 pg/ml; $p=0.243$), showing no statistical significance with controls.

The average TNF-a level in these patients is 171.3 pg/ml with a standard deviation of 26.7 pg/ml, and its standard error is 7.1 pg/ml. It was indicated by Wang et al. (2020) and Li et al. (2022) that TNF-a levels do not significantly change among individuals who are infected with Echinococcus. The lack of alterations is thus similar to what we have found presently.

Levels of Cytokines Depending on Age Among Study Groups

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	
	TNF-a pg/ml	< 40 year	18	147.3	36.4	8.6	0.002*
		> 40 year	15	182.5	16.1	4.2	
Healthy Control	Age (year)	< 40 year	34	27.6	10.7	1.8	0.001**
		> 40 year	33	52.3	7.3	1.3	
	TNF-a pg/ml	< 40 year	34	38.0	18.7	3.2	0.822
		> 40 year	33	39.2	22.3	3.9	

Table 4 explain the levels of certain cytokines (IL-6, TNF- α , IL-1 β , and IL-4) 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 TNF- α levels are higher significance in patients < 40 years old compared to those > 40 years old ($p = 0.001$, $p = 0.002$, $p = 0.001$ and $p = 0.001$) 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 Salem & El-Ghareeb, (2019) and Nian et al. (2022), showed that TNF-a levels were significantly higher in patients with Echinococcus multilocularis infection compared to controls ($p < 0.01$).

The Comparison between the Levels of Cytokines According to the Number of Cysts

Table 5. The Comparison between the Levels of Cytokines According to the Number of Cysts

		Mean	SD	SE	p-value
TNF-a pg/ml	One cyst	152.50	38.87	9.43	0.237 ns
	Two cysts	164.65	20.72	10.36	
	Three or more	178.06	23.70	6.84	

Table 5 showed that the levels of all cytokines that included in the current study TNF- α 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. On a similar note, Kumar et al., (2015) and Beyhan et al. (2022) found that TNF- α levels differ greatly between those afflicted with Echinococcosis and healthy individuals. They did not however make any mention of whether this discrepancy could be tied back to the quantity of cysts present. Hussain et al. (2020) and Halim et al. (2022) established that the levels of TNF-a are directly proportional to the severity of Echinococcosis symptoms but did not present the values of the mean and standard deviation for TNF-a levels.

Correlation Analysis Among Different Cytokines in Serum of Echinococcosis Patients

Table 6. Correlation Analysis Among Different Cytokines in Serum of Echinococcosis Patients

		Age (year)	TNF-a pg/ml
TNF-a pg/ml	R	0.812**	--
	p-value	<0.001	
	N	33	33

The table 6 presents the results of a correlation analysis (Pearson correlation coefficient) (R) between different cytokines. The correlations ranges 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 (TNF- α)($r > 0.8$, $p < 0.001$). This suggests that they are often co-regulated and may be involved in similar biological processes. Study of Ahmed et al. (2015) and Tilioua et al. (2020) found that TNF- α were significantly elevated in patients with hydatid cysts, and that these cytokines were correlated with disease severity ($r = 0.65$, $p < 0.01$).

Conclusion

The conclusion is the prevalence of hydatid disease in Al-Najaf Al-Ashraf in this study was 33%. Levels of the cytokines TNF- α 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. TNF- α levels showed significant differences between patients with 1, 2, 3, or more cysts.

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