*Original Research*

# EVALUATION OF TNF-A CONCENTRATION LEVEL IN PATIENTS INFECTED WITH HYDATID CYSTS IN AL-NAJAF HOSPITALS

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## ABSTRACT

**Background:** Human echinococcosis is a zoonotic disease caused by Echinococcus granulosus, the etiological agent of cystic echinococcosis (CE). **Aim:** This study aims to determine the epidemiological prevalence of Echinococcus granulosus in patients with hydatid cysts and evaluate serum TNF-α levels associated with echinococcosis, as well as the correlation between these levels and disease progression. **Methods:** Radiological examinations were performed to diagnose Echinococcus granulosus by identifying echinococcal cysts. The study included patients of all ages and both sexes from Al-Sader Medical City, Al-Hakeem General Hospital, Al-Haidarya General Hospital, and Al-Hayat Hospital. The study period was from October 2023 to the end of January 2024. **Results:** The mean TNF-α level in patients was 163.27 pg/ml, significantly higher than the mean level of 38.58 pg/ml in controls (p-value < 0.001). **Conclusion:** The prevalence of hydatid disease in Al-Najaf Al-Ashraf was found to be 33%. TNF-α levels are notably higher in patients with Echinococcus granulosus who are under 40 years of age compared to those over 40 years of age.

**Keywords**: *Echinococcus, Tumor Necrosis Factor Alpha, and  Hydatid Cyst.*

## 1. INTRODUCTION

In humans, this causes a disease called echinococcosis. The three types of echinococcosis are cystic echinococcosis caused by e. granulosus, and alveolar echinococcosis caused by e. Multilocularis and polycystic echinococcosis are caused by e. Vogeli or e. oligarchs. A worm's incubation period is usually up to 50 years (Marija and Pramodhini, 2022).

A 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).

TNF-α is a pro-inflammatory cytokine that is produced most by macrophages through several mechanisms (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 IFN-γ where IFN-γ will produce TNF-α as an anti-microbe(Fasano *et al.*, 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 (Liu *et al.*, 2020). IL-10 produces a lower degree of immunosuppression in unilocular disease that is more localized (Fasano *et al.*, 2022).

The study aims to determine the epidemiological prevalence of Echinococcus granulosus in patients infected with hydatid cysts, evaluate serum TNF-α associated with echinococcosis, and assess the correlation between them and the development of the disease.

## 2. MATERIALS AND METHODS

### 2.1 Patients Group

A case-control study design was chosen for patients infected with hydatid cysts (33 participants).

A radiological examination will be performed for these patients to diagnose the 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 patients from both sexes (males and females). Every patient was reported through a specifically prepared questionnaire, which included name, gender, age, living, site of cysts, and duration of disease. at al-Sader medical city, al-Hakeem general hospital, al-haidarya general hospital, and al-hayat Hospital.

### 2.2 Control group

The control group was 50 people not suffering from echinococcosis disease. The control group was used only for comparing parameters. The control samples were approximately similar to the sample patients in terms of number, ratio of age, and place of living in the countryside and city. Also, ask a special question sheet for the control samples where blood was drawn from a vein to measure immunological parameters TNF-α.

### 2.3 Serum collections

Five ml of venous blood was withdrawn from each subject by vein puncture using a sterile syringe with needle gauge 23, then the blood sample was transferred into coagulate gel tubes, then centrifuged for 5 minutes at 4000 (rpm) to separate serum were transferred to another sterile eppendorf tubes, labeled with a serial number together with the patient name, and frozen at (-20°c) until used.

The ELISA technique used the quantitative sandwich enzyme immunoassay technique to measure interleukin 6 in the patient's serum. The test was performed according to the company's 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.

### 2.3. Statistical analysis:

All data were statistically analyzed by IBM SPSS 26 (SPSS Inc., Chicago, IL). Frequencies and percentages with the use of the chi-square test reported nominal data. The normal distribution used the Kolmogorov-Smirnov test. Numerical data were expressed as mean ± standard deviation (SD), an independent t-test between two groups, an ANOVA test, and post hoc for comparison among groups. Pearson correlation was used. The receiver operation curve (ROC) is used to determine the area under the curve (AUC) with a confidence interval (95% CI) of TNF-a for discriminating between patients and controls by obtaining the cut-off point. Differences with p values < 0.05 were considered to be statistically significant. The significant level was a p-value <0.05 Sullivan, 2022; Al Khafaji and Sharba, 2022).

## 3. RESULTS AND DISCUSSION:

### 3.1. Results

#### 3.1.1. Demographic characteristics of study groups

Table 1 presents the demographic characteristics of two groups: echinococcosis patients and control, with 100 individuals. The chi-squared test results indicate 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 no significant differences between the two groups regarding age, sex, education level, or job.

#### 3.1.2. Cytokine level 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, tumor necrosis factor-alpha.

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, significantly higher than the Mean level of 38.58 pg/ml in controls (p-value< 0.001).

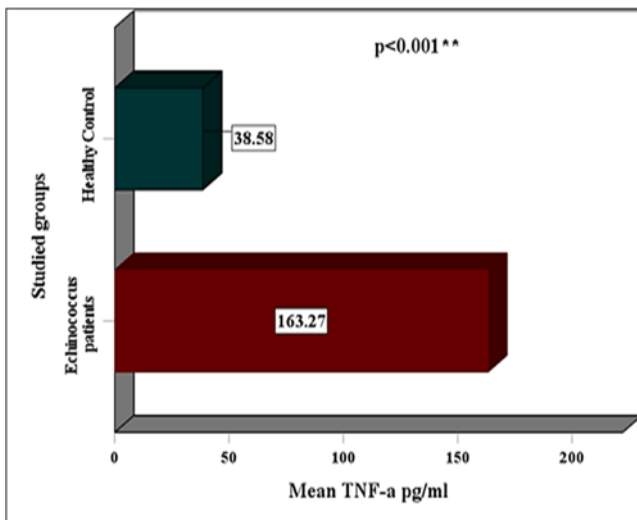**Figure 1:** TNF-α levels in study groups of echinococcosis patients and controls.

*Significant* differences at p-value **<0.01

### 3.1.3. Levels of cytokines depending on sex among study groups

Table 3 explains the results of a study comparing the levels of certain inflammatory cytokines (TNF-α) 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.

### 3.1.4. Levels of cytokines depending on age among study groups

Table (4) explains the levels of certain cytokines ( tnf-α) in the serum of patients with echinococcus infection and control individuals depending on age (< 40 years and > 40 years). The results were as follows:

Among echinococcus patients, TNF-α levels are significantly higher 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 these cytokines may be associated with echinococcosis infection. The study also found no significant differences in cytokine levels among control individuals between individuals < 40 years old and those > 40 years old.

### 3.1.5. The comparison between the levels of cytokines according to the number of cysts.

Table (5) shows that the levels of all cytokines included in the current study TNF-α did give light difference 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.

### 3.1.6. Correlation analysis between TNF-a and Age in the serum of Echinococcosis patients

Table 6 presents the results of a correlation analysis (Pearson correlation coefficient) (R) between TNF-a cytokine. The correlations range from -1 (perfect negative correlation) to 1 (perfect positive correlation). A strong positive significant correlation between TNF-a and age (r=0.812, p<0.001). The p-value indicates the probability that the observed correlation is due to chance.

### 3.1.7. ROC analysis of TNF-a to predict risk cytokine in Echinococcosis patients

Table 7 and Figure 7 display the results of AUC and ROC curve analysis of cytokine (TNF-α), statistically significant relationships of TNF-a with Echinococcosis patients by obtained of AUC:0.997, 95%CI: 90.56-1.003, P<0.001.
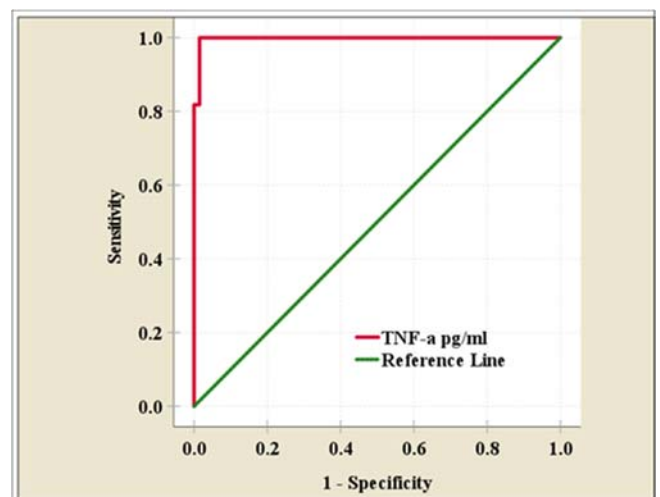


**Figure 7:** ROC Analysis of TNF-a cytocine

### 3.2. Discussion

As for the age, the mean age of the patients is 37.24 years, the standard error (SE) is 2.35, and the mean age of the control group is

39.78 years and SE 1.81. As shown in the table, the age is also presented in separate columns as < 40 and greater than 40 years; the age distribution demonstrates that both groups' age distribution is almost similar. Meanwhile, a study in Iraq also took place from 2018 to 2019, indicating the prevalence of echinococcosis among the population of different ages; the most affected group is 20-40 years old (Al-khazraji *et al.*, 2019). The mean age of the current study is also supported by a study conducted in Iraq in 2015-2018, and it depicted a mean age of 36.4 years (Al-Talib *et al.*, 2019).

From the above similarities, the mean age for the same duration of patients treated and control group patients was 40.2 years. In this study, the current one, fewer 40-year-old people were identified as compared to the control group of 54.5% and 50.7%. Abdul-Rahman *et al.*, 2018, note that from 2016 to 2017, echinococcosis infections were significantly higher in Iraq than those reported in younger individuals. Similarly, the patient and control groups have slightly higher percentages of male patients than the control groups. The current findings are consistent with earlier studies in Iraq from 2015 to 2024 (Abdul-Rahman *et al.*, 2018; Hassan *et al.*, 2019; Al-Abadi *et al.*, 2021).

Although the percentage of university graduates is higher among patients, there is no significant difference. This finding is analogous to several studies conducted in Iraq between 2015 and 2024. the researchers also found no statistically significant association between patient education level and Echinococcus. Zhang *et al.*, 2017 study found that TNF-α levels were significantly higher in patients with chronic inflammatory diseases than healthy controls. Kim *et al.* 2022 study reported that TNF-α levels were elevated in patients with psoriasis, an autoimmune disease characterized by skin inflammation. This study also found that TNF-α levels were associated with disease severity and response to treatment. Chen *et al.*, 2018 study found that TNF-α levels were associated with disease activity and severity in patients with rheumatoid arthritis. It was indicated by Wang *et al.* (2020) and Li *et al.* (2022) that TNF-α 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. A study by Salem and El-ghareeb (2019) showed that TNF-α levels were significantly higher in patients with Echinococcus multilocularis infection compared to controls (p< 0.01). Another study introduced by

Wang and Zhang (2022) found that TNF-α levels were elevated in patients with Echinococcus granulosus infection compared to controls (p < 0.05). This agrees with our current results. On a similar note, Kumar *et al.* (2015) found that TNF-α levels differ greatly between those afflicted with echinococcosis and healthy individuals; they did not mention whether this discrepancy could be tied back to the number of cysts present.

Hussain *et al.* (2020) established that the levels of TNF-α are directly proportional to the severity of echinococcosis symptoms but did not present the mean and standard deviation values for TNF-α levels.

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Similarly, the mean TNF-α 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-α 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-α 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.

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directly proportional to the severity of echinococcosis symptoms but did not present the mean and standard deviation values for TNF-α levels.

An *et al.* (2017) and Yao *et al.* (2017) reported elevated TNF-a levels in cystic echinococcosis patients compared to healthy controls; however, Yao's study found a more pronounced correlation. The research conducted by Zhang *et al.* in 2020 demonstrated that the levels of TNF-a were related to the severity of the disease and how patients respond to treatment in cases of alveolar echinococcosis.

# 4. CONCLUSIONS:

The study found that the prevalence of hydatid disease in al-Najaf al-Ashraf was 33%. It was observed that levels of the cytokine TNF-α are more significant in patients with Echinococcus granulosus who are under 40 years old than in those over 40 years old. There is evidence that individual differences in cytokine levels may impact the development or susceptibility to the disease. Furthermore, TNF-α levels showed significant variances between patients with 1, 2, 3, or more cysts.

# 5. Declarations

## 5.1. Study Limitations

The relatively small sample size, the lack of long-term follow-up, and the single-center nature of the study are some of the limitations of this study.

## 5.2. Acknowledgements

## 5.3. Funding Source

## 5.4. Competing Interests

The authors declare that they don`t have any potential conflict of interest.

## 5.5. Open Access

# 6. Human and Animal-related studies

## 6.1. Ethical Approval

Ethical Approval was obtained from the Council of Al-Furat Al-Awsat University, College of Health and Medical Technology, following the Helsinki Declaration (Number 36170, dated 12/09/2023)

## 6.5. Informed Consent

The patients were informed about the study, and their permission was obtained to use the anonymized information.

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**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 |
| | 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 |
| | freelancer | 19 | 57.6% | 39 | 58.2% | 58 | 58.0% | 0.907 ns |
| | student | 8 | 24.2% | 14 | 20.9% | 22 | 22.0% | |
| total | | 33 | 100.0% | 67 | 100.0% | 100 | 100.0% | |

ns: non- significant differences

**Table 2:** cytokines levels in study groups of echinococcosis patients and controls

| Groups | | Mean | SD | SE | p-value |
|---|---|---|---|---|---|
| TNF-α pg/ml | Patients | 163.27 | 33.65 | 5.86 | <0.001** |
| | Control | 38.58 | 20.39 | 2.49 | |

**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-α 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-α 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 4:** levels of cytokines depending on age< 40 years and  > 40 years among study groups

| Studied groups | Age group | | N | Mean | SD | SE | p-value |
|---|---|---|---|---|---|---|---|
| **Echinoco ccus 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 | |

Significant differences at p-value *<0.05, p **<0.01.

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

| | | Mean | SD | SE | p-value |
|---|---|---|---|---|---|
| **TNF-α pg/ml** | One Cyst | 152.50 | 38.87 | 9.43 | |
| | Two Cysts | 164.65 | 20.72 | 10.36 | 0.237 ns |
| | Three or More | 178.06 | 23.70 | 6.84 | |

significant differences at p-value *<0.05, p**<0.01

**Table 6:** Correlation analysis between TNF-a and Age  in the serum of Echinococcosis patients

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

**Table 7:** AUC and ROC analysis of TNF-a to predict risk in Echinococcosis patients

| Predictors | Area | SE | p-value | 95%CI |
|---|---|---|---|---|
| TNF-α pg/ml | 0.997 | 0.003 | <0.001** | 90.56-1.003 |

Significant at **p<0.01. 95%CI: Confidence interval. SE: standard error. AUC: Area under the curve.